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Identification of *Heterorhabditis* (Nematoda : Heterorhabditidae) from California with a new species isolated from the larvae of the ghost moth *Hepialis californicus* (Lepidoptera : Hepialidae) from the Bodega Bay Natural Reserve

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Summary – Classical taxonomy together with cross-breeding tests and random amplified polymorphic DNA (RAPD's) were used to detect morphological and genetic variation between populations of *Heterorhabditis* Poinar, 1975 from California. A new species, *Heterorhabditis hepialius* sp. n., recovered from ghost moth caterpillars (*Hepialis californicus*) in Bodega Bay, California, USA is herein described and illustrated. This is the eighth species in the genus *Heterorhabditis* and is characterized by the morphology of the spicules, gubernaculum, the female's tail, and ratios E and F of the infective juveniles. Information on its bionomics is provided.

Résumé – *Identification d'Heterorhabditis (Nematoda : Heterorhabditidae) de Californie dont une nouvelle espèce isolée de larves d'Hepialius californicus (Lepidoptera : Hepialidae) provenant de la réserve naturelle de la baie de Bodega* – Les méthodes de taxonomie classique de même que des essais de croisement et l'amplification au hasard de l'ADN polymorphique (RAPD) ont été utilisés pour détecter les différences morphologiques et génétiques entre populations d'*Heterorhabditis* Poinar, 1975 provenant de Californie. Une nouvelle espèce, *Heterorhabditis hepialius* sp. n., présente dans des chenilles d'*Hepialis californicus* provenant de la baie de Bodega (Californie, États-Unis d'Amérique) est décrite et illustrée. Représentant la huitième espèce du genre, *H. hepialius* sp. n. est caractérisé par la morphologie des spicules, du gubernaculum et de la queue de la femelle ainsi que par les rapports E et F chez les juvéniles infestants. Des données sur sa biologie sont également fournies.

Key-words : California, cross-breeding tests, Hepialidae, *Hepialis californicus*, Heterorhabditidae, *Heterorhabditis hepialius*, Lepidoptera, Nematoda, RAPD.

Entomopathogenic nematodes of the family Heterorhabditidae are considered effective biological control agents of insect pests (Gaugler & Kaya, 1990). With the increasing commercial use of these nematodes as biological control agents, many new isolates have been recovered from soil of natural habitats in many parts of the world, including Europe (Mraček, 1980; Burman *et al.*, 1986; Deseo *et al.*, 1988; Vanninen *et al.*, 1989; Hominick & Briscoe, 1990; Griffin *et al.*, 1991), North America (Akhurst & Brooks, 1984; Hara *et al.*, 1991), Australia (Akhurst & Bedding, 1986), and South America (Stock, 1993). However, most of these isolates remain unidentified at the specific level.

At present, seven species belonging to the genus *Heterorhabditis* have been described: *H. bacteriophora* Poinar, 1976; *H. megidis* Poinar, Jackson & Klein, 1987; *H. zealandica* (Poinar, 1990); *H. indicus* Poinar, Karunakar & Hastings, 1992; *H. argentinensis* Stock, 1993; *H. hawaiiensis* Gardner, Stock & Kaya, 1994, and *H. brevicaudis* Liu, 1994.

While sampling populations of pathogens of soil-inhabiting insects in northern and southern California, several *Heterorhabditis* isolates were recovered from insect hosts and soil samples. Material sent to the senior author was studied and the presence of a new *Heterorhabditis* species was determined which is herein described.

Materials and methods

ISOLATION OF NEMATODES

Several populations of *Heterorhabditis* were recovered from both soil and insect hosts while conducting a survey of soil-dwelling insect pathogens in northern and southern California (Fig. 1). The survey was mainly orientated towards the collection of white grubs (Coleoptera : Scarabeidae), white fringed beetles (Coleoptera : Curculionidae) and ghost moth caterpillars (Lepidoptera : Hepialidae).



Fig. 1. Collection sites of Californian *Heterorhabditis* isolates. 1: Moraga; 2: Balboa Park; 3: Chino Hills; 4: Davis; 5: Hercules; 6: Modesto; 7: Oakmont; 8: Temecula; 9: Bodega Bay.

Nematodes were recovered from both insects and soil-samples taken at different localities throughout California (Table 1).

Infected insects collected from the field were placed in White traps until emergence of nematodes. Soil samples were baited with *Galleria mellonella* larvae as described by Bedding and Akhurst (1975) to test for the presence of entomopathogenic nematodes.

In all cases the recovered nematodes were exposed to fresh *G. mellonella* larvae to confirm pathogenicity and complete Koch's postulates. Infective juveniles were re-recovered using White traps and harvested in a cold-room at 10 °C.

Endosymbiotic bacteria *Photorhabdus luminescens* Poinar & Thomas, 1976 were isolated from the intestine of surface sterilized infective juveniles following Akhurst (1980) and the primary forms of the bacteria were maintained in NBTA plates (Akhurst, 1986).

CROSS-BREEDING TESTS USING *HETERORHABDITIS* SPP.

Crosses were carried out on lipid agar plates (Dix *et al.*, 1992). Second generation males and females were

Table 1. Isolates of *Heterorhabditis* from California: location, habitat, site code and association with insect host are indicated.

| Location | Habitat | Site Code | Association with insect hosts |
|-------------------------|----------------------------|-----------|--|
| Balboa Park (San Diego) | park grassland | AGA | white grub (<i>Cylocephala pasadane</i>) |
| Bodega Bay (Sonoma) | inland edge of dunes | BOD | ghost moth (<i>H. californicus</i>) |
| Chino Hills (Riverside) | hillside managed landscape | CHI | white fringed beetle (<i>Graphognathus</i> sp.) |
| Davis (Yolo) | baseball field | DAV | white grub (<i>C. hirta</i>) |
| Hercules (Contra Costa) | golf course turf | HER | white grub (<i>C. hirta</i>) |
| Moraga (Contra Costa) | golf course (turf) | AGA | white grub (<i>C. hirta</i>) |
| Modesto (Stanislaus) | vineyard | MOD | none found |
| Oakmont (Napa) | golf course (turf) | OAK | white grub (<i>C. hirta</i>) |
| Temecula (Riverside) | golf course (turf) | TEM | none found |

dissected from *G. mellonella* larvae which were previously incubated at 25 °C for 6–7 days post infection with infective juveniles of the appropriate *Heterorhabditis* isolate. Insect cadavers were dissected in Ringer's solution using a stereomicroscope at 50× magnification. Females with immature gonads were selected for the cross-breeding tests.

Lipid agar plates (5 cm diameter) were prepared by the method of Dunphy and Webster (1989) and inoculated with the bacterial isolate of the appropriate nematode strain and incubated at 30 °C for 2 days. For each cross five virgin females and five males of the appropriate isolate were placed on each lipid plate and incubated at 25 °C; 3 days later an additional five males were added to each plate.

In successful crosses, progeny were visible after 2–3 days. Controls were always set up for each cross; virginity/self fertility tests: five virgin females (without males) were placed onto lipid agar plate; mating test/self cross: five virgin females and five males of the same isolate were placed on a single lipid agar plate and additional five males were added to the plate 3 days later.

The results of the crosses between different isolates were taken as valid only if there were no progeny in the virginity tests and if there were progeny in the self-crosses.

RAPD ANALYSIS

Methods of randomly amplified polymorphic DNA extraction and RAPD analysis followed Gardner *et al.* (1994). Operon[®] random primers (OPA-02, OPA-05, OPA-11, OPA-16, OPB-04) of ten bases long (Table 2) were used for all reaction experiments with an annealing temperature of 35 °C. Purified DNA from nematode genome was subjected to PCR reaction. PCR products were resolved by electrophoresis using a 1.7 % horizontal agarose gel, and imaged by staining with ethidium bromide.

MORPHO-BIOMETRICAL STUDIES

Nematodes recovered from *Galleria mellonella* larvae were fixed in TAF and cleared in glycerol. Infective juveniles and first generation adults of the *Heterorhabditis* isolate from Bodega Bay were also recovered from *Hepialis californicus* caterpillars. Quantitative measurements were made using a Leitz Ortholux II microscope equipped with an ocular micrometer and Jandel JAVA[®] software with a high resolution video camera. Drawings were made with the aid of a drawing tube.

To test the robustness of the morphometric variables, a multivariate analysis (canonical discriminant analysis) was performed on males and infective juveniles (Stock *et al.*, 1995).

Results

CROSS-BREEDING TESTS

According to the results obtained by the morphological and morphometric observations, the cross-breeding tests were done only between *H. bacteriophora* (NC1 strain) and the nine Californian isolates. Crosses were only carried out in lipid agar plates.

From all the possible combinations, only fourteen pairs of crosses among the Californian isolates were randomly selected for the evaluation of this part of the experiment (Table 3). Isolates from Moraga (AGA), Balboa Park (BAL), Chino Hills (CHI), Hercules (HER), Modesto (MOD), Oakmont (OAK) and Temecula (TEM) resulted in fertile progeny when crossed with each other and with *H. bacteriophora* (NC1 strain) but the Bodega Bay isolate (H.h.) did not hybridize either with other Californian isolates or with *H. bacteriophora* (NC1 strain).

RAPD ANALYSIS

Throughout the present study RAPD reactions were always duplicated and care was taken to ensure consistency in DNA banding patterns between replicates and between separate experimental runs. Two of the RAPD profiles obtained from the thirteen *Heterorhabditis* isolates/species tested are shown in Figure 2.

Table 2. Oligonucleotide primers used in the present study (see Gardner *et al.*, 1994).

| Reference | Primer sequence (5' to 3') |
|-----------|----------------------------|
| OPA-02 | CAGGCCCTTC |
| OPA-05 | AGGGGTCTTG |
| OPA-16 | AGCCAGCGAA |
| OPB-04 | GGACTGGAGT |
| OPB-11 | GTAGACCCGT |

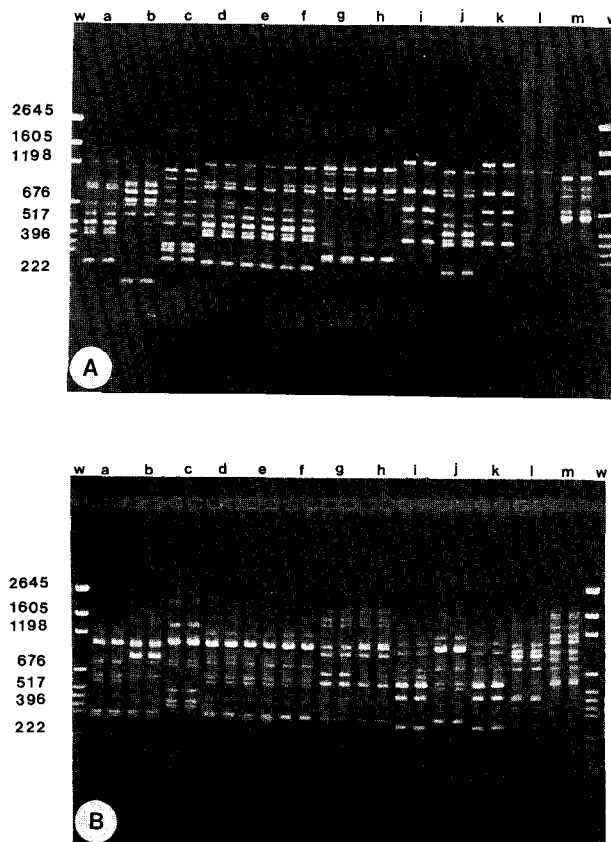


Fig. 2. RAPD profiles of thirteen isolates/species of *Heterorhabditis*. Banding patterns yielded by two different primers: A: OPA-05; B: OPA-11. For each presumptive isolate/species, the sample was duplicated, thus there are two lanes on the gel for each isolate/species. From left: w, ladder molecular weight marker (lane 1); a: *H. bacteriophora* (NC1 strain); b: *H. argentinensis* (lanes 2-3); c: *H. hepialius* sp. n. (lanes 4-5); d: *H. bacteriophora* (DAV strain) (lanes 6-7); e: *H. bacteriophora* (BAL strain) (lanes 8-9); f: *H. bacteriophora* (CHI strain) (lanes 10-11); g: *H. hawaiiensis* (HK3) (lanes 12-13); h: *H. hawaiiensis* (MG strain) (lanes 14-15); i: *H. megidis* (strain) (lanes 16-17); j: *H. bacteriophora* (OAK strain) (lanes 18-19); k: uncharacterized *Heterorhabditis* from Tasmania (lanes 20-21); l: *H. zealandica* (lanes 22-23); m: uncharacterized *Heterorhabditis* from Azores (lanes 24-25).

Table 3. Results of cross-breeding experiments among *Heterorhabditis* isolates from California and with *H. bacteriophora* (NC1 strain).

| Female * Male * | AGA | BAL | CHI | DAV | HER | MOD | OAK | TEM | H.h. (BOD) | H.b. |
|--------------------|-----|-----|-----|-----|-----|-----|-----|-----|---------------|------|
| AGA | + | + | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 |
| BAL | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CHI | 0 | 0 | + | + | + | 0 | 0 | 0 | 0 | - |
| DAV | 0 | 0 | + | + | 0 | 0 | 0 | 0 | 0 | + |
| HER | 0 | 0 | 0 | 0 | + | + | 0 | 0 | 0 | 0 |
| MOD | 0 | 0 | 0 | 0 | + | + | 0 | 0 | 0 | 0 |
| OAK | 0 | 0 | 0 | 0 | 0 | 0 | + | + | 0 | + |
| TEM | 0 | 0 | 0 | 0 | 0 | 0 | + | + | 0 | 0 |
| H.h. (BOD) | - | 0 | - | 0 | 0 | 0 | 0 | 0 | + | - |
| H.b. | 0 | 0 | 0 | + | 0 | 0 | + | 0 | - | + |

References : * Origin of *Heterorhabditis* isolates/species : AGA, Moraga; BAL, Balboa Park; CHI, Chino Hills; DAV, Davis; CHI, Chino hills; HER, Hercules; MOD, Modesto; OAK, Oakmont; TEM, Temecula; H.h. (BOD), *H. hepialus* sp. n.; H.B., *H. bacteriophora*. +, Progeny; -, No progeny; 0, No cross-breeding test.

A similarity matrix based on Jaccard's coefficient of shared RAPD bands among the different isolates/species shows that the *Heterorhabditis* population from Bodega Bay is distinct, sharing a maximum of 40 % of the RAPD bands (Table 4), and this is with an isolate of an undescribed nematode from Tasmania. Similarity coefficients of pairwise comparisons were used for nearest neighbour hierarchical cluster analysis.

This analysis shows the relatedness of the Californian isolates (except the Bodega Bay isolate) with *H. bacteriophora* (NC1 strain) and that there is consistency with the results obtained by the morphological study (Fig. 3).

MORPHO-BIOMETRICAL STUDIES

From a total of nine isolates from California, eight were identified as *Heterorhabditis bacteriophora* Poinar, 1975 by means of morphology and associated morphometrics.

Results from the multivariate analysis showed that, for both males and infective juveniles, the Bodega Bay isolate stands apart from the other Californian *Heterorhabditis* and also from *H. bacteriophora* (NC1 strain). The rest of the Californian *Heterorhabditis* were indistinguishable between each other and with *H. bacteriophora* (NC1) (Stock *et al.*, 1995). Comparative morpho-biometrical studies with other *Heterorhabditis* species showed that the Bodega Bay isolate was an undescribed species of the genus *Heterorhabditis*, and its description follows.

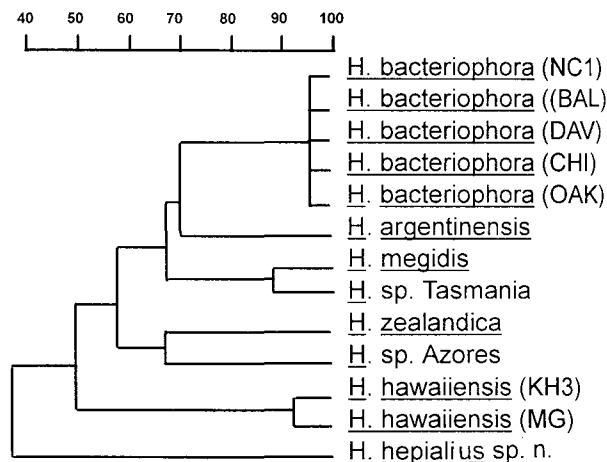


Fig. 3. Relationships of thirteen isolates/species of *Heterorhabditis*. The dendrogram was generated from the Jaccard's coefficient of similarity obtained from presence/absence of a total of 32 RAPD markers generated by the random primers. The horizontal distance between the clusters is proportional to similarity.

Heterorhabditis hepialus * sp. n. (Fig. 4)

MEASUREMENTS

Hermaphroditic females, males, amphimictic females and third -stage infective juveniles : see Table 5.

* Named after its natural host *Hepialis californicus*.

Table 4. Jaccard's similarity coefficient of shared amplified polymorphic DNA (RAPD) bands among thirteen isolates and species of *Heterorhabditis*. Two different collection localities of *H. hawaiiensis* were included to test for consistency of genetic identity.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|--|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1. <i>H. bacteriophora</i> (NC1 isolate) | 1.000 | | | | | | | | | | | | |
| 2. <i>H. argentinensis</i> | 0.750 | 1.000 | | | | | | | | | | | |
| 3. <i>H. hepialius</i> sp. n. | 0.313 | 0.363 | 1.000 | | | | | | | | | | |
| 4. <i>H. bacteriophora</i> (BAL isolate) | 0.969 | 0.781 | 0.344 | 1.000 | | | | | | | | | |
| 5. <i>H. bacteriophora</i> (DAV isolate) | 0.969 | 0.781 | 0.344 | 1.000 | 1.000 | | | | | | | | |
| 6. <i>H. bacteriophora</i> (CHI isolate) | 0.969 | 0.781 | 0.344 | 1.000 | 1.000 | 1.000 | | | | | | | |
| 7. <i>H. hawaiiensis</i> (KH3) | 0.536 | 0.500 | 0.375 | 0.531 | 0.531 | 0.531 | 1.000 | | | | | | |
| 8. <i>H. hawaiiensis</i> (MG) | 0.536 | 0.500 | 0.375 | 0.531 | 0.531 | 0.531 | 0.531 | 1.000 | | | | | |
| 9. <i>H. megidis</i> | 0.656 | 0.656 | 0.346 | 0.688 | 0.688 | 0.688 | 0.469 | 0.469 | 1.000 | | | | |
| 10. <i>H. bacteriophora</i> (OAK isolate) | 0.969 | 0.781 | 0.344 | 1.000 | 1.000 | 1.000 | 0.531 | 0.531 | 0.68 | 1.000 | | | |
| 11. <i>H. sp.</i> Tasmania | 0.719 | 0.719 | 0.406 | 0.688 | 0.688 | 0.688 | 0.531 | 0.531 | 0.813 | 0.688 | 1.000 | | |
| 12. <i>H. zealandica</i> | 0.625 | 0.750 | 0.348 | 0.594 | 0.594 | 0.594 | 0.625 | 0.625 | 0.656 | 0.594 | 0.844 | 1.000 | |
| 13. <i>H. sp.</i> Azores | 0.750 | 0.688 | 0.375 | 0.781 | 0.781 | 0.781 | 0.625 | 0.625 | 0.594 | 0.781 | 0.594 | 0.688 | 1.000 |

DESCRIPTION

Adults: Head truncated, or slightly rounded. Six distinct protruding lips surrounding the mouth with six papillae. Amphids inconspicuous. Cheilorhabdions represented as refractile areas; prohabdions, mesorhabdions and telorhabdions reduced. Oesophagus without metacarpus, but with a poorly developed isthmus and a basal bulb that lacking a valve. Nerve ring located at the beginning of the isthmus. Excretory pore opening usually posterior to nerve ring. Location of the excretory pore opening in relation to the oesophagus length (ratio D) 1.0 and 0.9 in first generation hermaphrodites and second generation adults (males and amphimictic females), respectively. Lateral fields and phasmids inconspicuous.

Female: Females with paired ovaries. Hermaphroditic females (first generation) with an ovotestis. Vulva with protruding lips near middle of body. Vulva of amphimictic females with slightly protruding lips. Tail of both females "pipette-shaped", with an accentuated swelling before the tail's tip that can vary in its length. Post-anal swelling is also presented in both females.

Male: Males with single testis, reflexed twice. Spicules paired, equal, separated and slightly curved. Manubrium rectangular shaped, well set off from calomus. Lamina without internal rib and with a very thin velum. Cuneiform-shaped gubernaculum half the length of spicules. Bursa peloderan attended by complement of nine

pairs of genital papillae with three pairs preanal and six pairs post-anal arranged in two sets of three. Pair 8 never reaching rim of bursal membrane.

Infective-stage juvenile (J3): Body slender, gradually tapering posteriorly. Lip region continuous. Cuticle with fine longitudinal striations. Mouth and anus closed. Nerve ring in middle of isthmus. Excretory pore located at level of basal bulb. Tail pointed.

TYPE LOCALITY

Bodega Bay (Bodega Marine Preserve) California, USA.

TYPE HOST

Ghost moth caterpillars, *Hepialis californicus* (L.) (Lepidoptera: Hepialidae).

TYPE MATERIAL

Holotype (male), allotype (amphimictic female), paratypes (five males, five hermaphroditic females five amphimictic females and five infective juveniles) in the Nematode Collection of the University of California, Davis, California, and USDA Nematode Collection, Beltsville, Maryland, USA.

DIAGNOSIS AND RELATIONSHIPS

Heterorhabditis hepialius sp. n. can be separated from other recognized *Heterorhabditis* species by morphological and DNA characters.

Morphologically the average length of *H. hepialius* sp. n. infective juveniles (574 μ m) is different from those of

Table 5. Measurements of different stages of *Heterorhabditis hepialius* sp. n. A: First generation hermaphroditic females; B: Second generation males; C: Second generation amphimictic females; D: Third-stage infective juveniles. (For each column: n = 30; all measurements in μm , except L in mm).

| Character/ratio | A | B | C | D |
|-----------------------------|------------------|------------------|------------------|------------------------|
| Body length (L) | 4.0-5.2 (4.6) | 0.8-1.0 (0.9) | 3.5-4.5 (3.9) | 0.540-0.600 (0.574) |
| Max. width (W) | 205-335 (263) | 65-98 (72) | 99-161 (125) | 34-39 (35) |
| Ant. end. excret. pore (EP) | 175-258 (203) | 102-131 (114) | 133-177 (154) | 84-112 (103) |
| Ant. end nerve ring (NR) | 117-161 (138) | 84-114 (101) | 99-135 (120) | 80-101 (95) |
| Oesophagus length (ES) | 190-223 (200) | 113-139 (124) | 150-183 (165) | 106-130 (118) |
| V | 45-50 (47) | - | 49-51 (50) | - |
| Tail length (TL) | 60-126 (98) | 37-49 (40) | 76-113 (89) | 49-60 (54) |
| Width at anus (WA) | 34-60 (44) | 21-30 (26) | 24-60 (31) | 9-14 (12) |
| Spicules length (SL) | - | 42-52 (47) | - | - |
| Spicules width (SW) | - | 4-7 (6) | - | - |
| Gubernac. length (GL) | - | 17-24 (21) | - | - |
| Gubernac. width (GW) | - | 1.5-2.2 (1.8) | - | - |
| Ratio A (L/W) | - | - | - | 5-7 (6) |
| Ratio B (L/ES) | - | - | - | 4-5 (4-5) |
| Ratio C (L/TL) | - | - | - | 9-12 (10) |
| Ratio D (EP/ES) | - | - | - | 0.79-0.98 (0.87) |
| Ratio E (EP/TL) | - | - | - | 1.0-2.0 (1.35) |
| Ratio F (W/TL) | - | - | - | 0.19-0.25 (0.22) |

H. megidis (768 μm), *H. zealandica* (685 μm), *H. argentinensis* (657 μm), *H. indicus* (528 μm) and *H. bacteriophora* (588 μm). However, the length range of *H. hepialius* sp. n. infective juveniles (540-600 μm) somewhat overlaps with those of *H. bacteriophora* (512-670 μm), *H. zealandica* (570-740 μm) and *H. indicus* (479-573 μm); but no overlapping occurs with those of

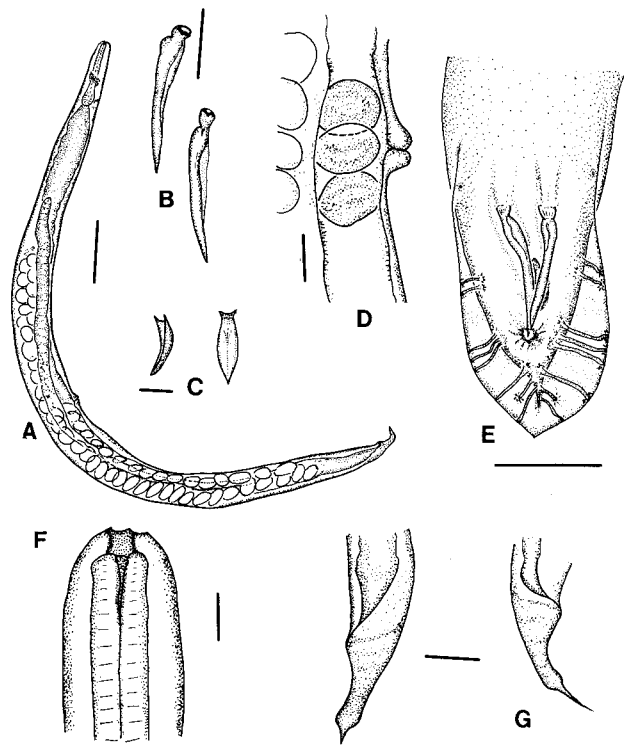


Fig. 4. *Heterorhabditis hepialius* sp. n. A: Second generation "amphimictic" female, whole body, lateral view; B: Spicules, lateral view; C: Gubernaculum, lateral and ventral view; D: Vulva, hermaphroditic female; E: Bursa, male; F: Anterior end, hermaphroditic female; G: Tail, hermaphroditic female, lateral view. (Bar equivalents: A = 100 μm ; B = 25 μm ; C, D, F = 10 μm ; E, G = 50 μm).

H. argentinensis (610-710 μm) and *H. megidis* (736-800 μm).

In terms of length the infective juveniles of *H. hepialius* sp. n. are practically indistinguishable with those of *H. hawaiiensis* (575 μm) and *H. brevicaudis* (572 μm). But the infective juveniles of the new species can be separated from these and the rest of the recognized *Heterorhabditis* species by the length of the tail (49-60 μm) and the value of ratio C (9-12) (Table 6).

Males can be separated from other *Heterorhabditis* species by the spicules which possess a rectangular or subtriangular manubrium and a lamina with a thin velum (not present in other *Heterorhabditis* species).

Hermaphrodites and amphimictic females of *H. hepialius* sp. n. can be distinguished from other *Heterorhabditis* by the shape of the tail (pipette-shaped), which possesses an accentuated swelling before the tail's tip.

Furthermore, PCR-RAPD analysis showed that only 40% of the bands evident in the gels were shared with other *Heterorhabditis* species/isolates (Table 4).

Table 6. Comparison of measurements of infective juveniles of *Heterorhabditis* species (All measurements in μm).

| Species | <i>H. bacteriophora</i> ^a | <i>H. zealandica</i> ^a | <i>H. megidis</i> ^a | <i>H. indicus</i> ^b | <i>H. hawaiiensis</i> ^c | <i>H. argentinensis</i> ^d | <i>H. hepialius</i> sp. n. | <i>H. brivicaudis</i> ^e |
|---------|--------------------------------------|-----------------------------------|--------------------------------|--------------------------------|------------------------------------|--------------------------------------|----------------------------|------------------------------------|
| L | 588 (512-670) | 685 (570-740) | 768 (736-800) | 528 (479-573) | 575 (506-631) | 657 (610-710) | 574 (540-600) | 572 (528-632) |
| W | 23 (18-31) | 27 (22-30) | 29 (27-32) | 20 (19-22) | 25 (21-28) | 31 (24-38) | 22 (20-24) | 23 (922-27) |
| AE-EP | 103 (87-110) | 112 (94-123) | 131 (123-142) | 98 (88-107) | 92 (79-103) | 107 (68-122) | 103 (84-112) | 111 (104-116) |
| AE-NR | 85 (72-93) | 100 (90-107) | 109 (104-115) | 82 (72-85) | 114 (95-132) | 95 (82-160) | 95 (80-106) | 101 (96-104) |
| AE-ES | 125 (100-139) | 140 (135-147) | 155 (147-160) | 117 (109-123) | 133 (115-181) | 132 (101-150) | 118 (106-130) | 124 (120-136) |
| TL | 98 (83-112) | 102 (87-119) | 119 (112-128) | 101 (93-109) | 90 (982-108) | 84 (70-105) | 54 (49-60) | 75.5 (68-80) |
| WA | | | | | | | 12 (9-14) | 8.8 (8.12) |
| R. A. | 25 (17-30) | 25 (24-26) | 26 (23-28) | 26 (25-27) | 23 (22-24) | 21 (18-25) | 23 (22-27) | |
| R. B. | 4.5 (4.0-5.1) | 4.9 (4.2-5.0) | 5.0 (4.6-5.9) | 4.5 (4.3-4.8) | 4.3 (3.5-4.4) | 5.0 (4.7-6.0) | 4.5 (4-5) | |
| R. C. | 6.2 (5.5-7.0) | 6.6 (6-6.7) | 6.5 (6.1-6.9) | 5.3 (4.5-5.6) | 6.4 (5.8-6.1) | 7.8 (6.7-8.7) | 10 (9-12) | 7.6 (6.6-8.6) |
| R. D. | 0.84 (0.76-0.92) | 0.80 (0.70-0.84) | 0.85 (0.81-0.91) | 0.84 (0.79-0.90) | 0.80 (0.56-0.68) | 0.78 (0.67-0.81) | 0.87 (0.79-0.98) | |
| R. E. | 1.12 (1.03-1.30) | 1.08 (1.03-1.09) | 1.10 (1.03-1.20) | 0.94 (0.83-1.03) | 1.2 (1.1-1.3) | 1.3 (0.9-1.1) | 1.35 (1.0-2.0) | 1.6 (1.5-1.8) |
| R. F. | 0.25 (0.22-0.36) | 0.25 (0.24-0.26) | 0.25 (0.23-0.28) | 0.20 (0.18-0.22) | 0.26 (0.25-0.27) | 0.35 (0.34-0.36) | 0.22 (0.19-0.25) | |

References : a, after Poinar, 1990; b, after Poinar *et al.*, 1992; c, after Gardner *et al.*, 1994; d, after Stock, 1993; e, after Liu, 1994.

ECOLOGY

Infective juveniles of *H. hepialius* sp. n. were found in the upper 20-30 cm of soil in the rhizosphere of bush lupine, *Lupinus arboreus* (Simms.) (Leguminosae, Papilionoideae). The soils of the type locality are sandy (sand, 89 %; silt, 8 %; clay, 2 %; organic matter 3-8 %; pH, 5-6).

This entomopathogenic nematode readily infects wax worms (*G. mellonella*) placed in soil collected from beneath the canopies of bush lupine. It also kills many subterranean caterpillars of the ghost moth that feed upon the roots of bush lupine. Both the host-plant and host-insect are native to the type locality, which is an ecological reserve (Bodega Marine Reserve, University of California). Small ghost moth caterpillars are parasitized by *H. hepialius* sp. n. during late spring, when they move through the soil in search of lupine roots. Cadavers of larger ghost moth caterpillars, orange-gold in col-

our and rife with this nematode, are found in crevasses on the exterior of lupine roots from late spring through summer. Caterpillars that succeed in boring inside the root, and plugging the hole with silk and frass, appear invulnerable to *H. hepialius* sp. n.

Discussion

Our results indicate that nematodes of the genus *Heterorhabditis* are present in California (Table 1).

As it has been stated by several authors (Smits *et al.*, 1991; Dix *et al.*, 1992; Griffin *et al.*, 1994), morphological characterization of *Heterorhabditis* species is difficult to interpret, especially for untrained biologists. However, the development of molecular techniques (such as allozyme electrophoresis, DNA restriction fragment length polymorphisms, DNA sequencing, and RAPD analysis) provides useful tools for diagnostic studies at the population and species level (Smits *et al.*, 1991; Gardner *et al.*, 1994). At the same time identification of

presumed biological species in the genus *Heterorhabditis* by cross-breeding of second generation amphimictic females has confirmed *H. bacteriophora* (Brecon strain), *H. megidis* and *H. zealandica* as distinct biological species (Dix et al., 1992).

The diagnostic morphological characters together with the molecular data (PCR-RAPD) obtained in the present study show that *H. hepialius* is unique and does not fit the description of currently recognized species of the genus *Heterorhabditis*, therefore it is considered a new species.

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