

***Rhabditis rainai* n. sp. (Nematoda: Rhabditida) associated with the Formosan subterranean termite, *Coptotermes formosanus* (Isoptera: Rhinotermitidae)**

Lynn K. CARTA^{1,*} and Weste OSBRINK²

¹ United States Department of Agriculture, ARS-BARC, Nematology Laboratory, Beltsville, MD 20705, USA

² United States Department of Agriculture ARS-SRRC, Formosan Subterranean Termite Research Unit, New Orleans, LA 70124, USA

Received: 11 April 2005; revised: 10 September 2005

Accepted for publication: 12 September 2005

Summary – *Rhabditis rainai* n. sp. is described from the gut and head of sick Formosan subterranean termites (*Coptotermes formosanus*) collected in New Orleans, LA, USA (isolate LKC20). *Rhabditis rainai* n. sp. is a hermaphroditic species with an unusual prerectum, four denticles per glottoid swelling, enlarged posterior stoma, three paired lip sectors, protruding posterior anal lip, and five ridges in the hermaphrodite lateral field. Males generated from starved bacterial cultures have three lateral field ridges and a notched peloderan bursa with only eight rays. Male spicules are distinctive with a round but truncated head, constricted neck, narrow shoulders, nearly straight blade with distal tenth upturned, and rounded tips supporting a curved ventral arch. Males of LKC20 were mated successfully with hermaphrodites cultured from a Fiji Islands soil (isolate PS1191). In subsequent crosses, male progeny were fertile as expected for conspecific populations. Differences in morphometrics of cultured specimens that were heat-killed or fixed is demonstrated. *Rhabditis rainai* n. sp. was compared to *R. blumi* and *R. adenobia*, but does not fit clearly within the current subgenera of *Rhabditis* or genera of Rhabditidae. Nematode-termite relationships and possible origin of *R. rainai* n. sp. with Formosan subterranean termites from Southeast Asia are discussed.

Keywords – description, facultative parasite, invasive species, morphology, morphometrics, *Oscheius*, *Rhabditis adenobia*, *Rhabditis blumi*, *Rhabditis silvatica*, SEM, taxonomy.

The Formosan subterranean termite *Coptotermes formosanus* Shiraki, 1909 is a non-endemic pest found in the southern United States. An unthrifty colony in Louisiana, USA was discovered to have juvenile and hermaphroditic adult nematodes inside the termite guts and heads. The nematode was cultured on bacteria and designated as population LKC20. Population PS1191, cultured from soil from the Fiji Islands, was compared morphologically and successfully mated with males of the termite population. Both populations were determined to be new and conspecific and are described here as *Rhabditis rainai* n. sp.

Materials and methods

Termites were collected by Weste Osbrink and isolated by Ashok Raina (Formosan Subterranean Termite Research Unit, USDA-ARS, New Orleans, LA, USA) in

July 2000 from a bucket trap at the University of New Orleans. Termite guts were dissected from live adults and placed on both water agar and nutrient agar where nematodes emerged. This population, referred to as LKC20, grew on a bacterium associated with the termite gut that flourished on nutrient agar and was then subcultured on NGM (nematode growth medium) agar plates with *E. coli* OP50 (Stiernagle, 1999). Population PS1191 was isolated on NGM plates of *E. coli* from soil particles from Kioa Island, Fiji, by Will Boorstein (California Institute of Technology, Pasadena, CA, USA) in September, 1992. The generation time of both populations was measured at 20 and 25°C from eggs of one generation to the second after daily plate transfers of the hermaphrodite. Brood size was determined by transfer of individual fourth-stage juvenile (J4) hermaphrodites to single plates over 5 days until no more eggs were laid. Images and measurements were made with a Zeiss Ultraphot III micro-

* Corresponding author, e-mail: cartal@ba.ars.usda.gov

scope (Carl Zeiss Inc., Jena, Germany, and Baltimore Instrument Co., Baltimore, MD, USA) equipped with differential interference contrast (DIC) optics. Drawings were made from photographs and camera lucida projections of live specimens. Image Pro-plus v. 3.0 for Windows® software (I-Cube Image Analysis/Processing, Crofton, MD, USA) was used for computer-generated images on specimens, either active, relaxed with 10 mM sodium azide (Bargmann & Avery, 1995), or killed by gentle heat. Live specimens from plates containing bacteria intended for measurement were either inactivated or killed after minimal passage through the flame of an alcohol lamp, or prepared for permanent slides after hot fixation in 4% formaldehyde before glycerol dehydration. Males were generated after at least two 9 cm diam. plates of hermaphrodites had bacteria depleted. A platinum worm-pick with bacteria was used to select between one and three rare males from among thousands of other starved hermaphrodites. These males were transferred to the centre of 6 cm diam. bacterial plates along with two to three J4 hermaphrodites to produce a high proportion of males after 3-4 days for further cross-population mating tests. Five males from these plates were mated with two J4 from other non-mated hermaphrodite plates on each of two 6 cm diam. plates. After 2 days the results of crosses were checked daily for male cross-progeny (modified from Wood, 1988). Males, hermaphrodites, and dauer juveniles were measured with an ocular micrometer. Statistics for mean, standard deviation, range, and Student's t-Test were made with an Excel® 2002 spreadsheet (Microsoft Corp., Redmond, WA, USA). Males were placed in 0.5% carmine dye (Kiontke & Sudhaus, 2000) and ringed with nail polish to enhance staining of phasmids (Premachandran *et al.*, 1988). Nematodes were prepared for scanning electron microscopy according to Golden (1990) and viewed under a Hitachi S570 SEM at 10 KV (Hitachi Ltd., Tokyo, Japan). Fixed specimens used to evaluate relationships were observed from the United States Department of Agriculture Nematode Collection (USDANC). These included slides of *Rhabditis adenobia* Poinar, 1971 (5-11-70 slide T 847p: four males and females) and *R. blumi* Sudhaus, 1974 (2-9-83 slide T 3064p, five agar culture females, and slide T3065p, five males), and live *R. blumi* DF5010 culture from the *Caenorhabditis* Genetics Centre (CGC). Stoma measurement was done according to Andrassy (1983) and terminology of its parts used after De Ley *et al.* (1995). Assignments of head sensory organs were made according to Riddle (1988) and Ward *et al.* (1975).

***Rhabditis rainai** n. sp.**
(Figs 1-4)

MEASUREMENTS

Type population LKC20 and population PS1191. For both fixed, and live, then heat-inactivated hermaphrodites see Table 1; for males, see Table 2; for dauer juveniles, see Table 3. For diagnostic measurements comparing old and new values for *Rhabditis adenobia*, *R. blumi*, *R. silvatica*, including *R. rainai* n. sp. range for all populations, see Table 4 for hermaphrodites and Table 5 for males.

DESCRIPTION

Hermaphrodite

Lip region high (3.5-4 × 18-27 μm lip base diam.), not offset. Six globose lip sectors fused pairwise (two dorsal sectors, right ventral and subventral sectors, left ventral and subventral sectors) separated by three deep grooves, one ventral and two dorso-lateral, with large apical inner labial sensillae, smaller subcuticular, lateral, outer labial and cephalic sensillae, and two oval, lateral amphidial apertures seen with SEM. Cheilostom not cuticularised. Isomorphic, metastegostom bases, each with four denticles, most easily viewed dorso-ventrally. Anterior gymnostom diam. near lips *ca* 72-79% of diam. of expanded stegostom base (5.1-6.6 μm). Collar extending 44-77% of buccal cavity length (buccal cuticle of stegostom plus gymnostom), or 35-61% of stoma length (buccal cavity plus lip length). Robust procorpus tapering outward toward centre, slightly swollen, but not curved and expanded like a median bulb. Procorpus *ca* twice length of isthmus, *ca* equal to isthmus and terminal bulb. Terminal bulb comprising 75% of isthmus length, with centrally positioned valvular apparatus anterior to duplex haustorium. Deirid located eight annules posterior to, and outside of, first two lateral lines, *ca* five annules (6-7 μm) anterior to level of excretory pore. Excretory pore 1.5 μm diam., anterior or posterior to terminal bulb in fixed specimens, at mid-isthmus to terminal bulb level in live specimens; cuticularised duct curving laterally inward before branching anteriorly and posteriorly. Lateral field composed of five ridges at midbody, with total of six to eight lines sometimes visible. Dorsal and ventral side cuticle

* The new species is named after Dr Ashok Raina who first isolated the nematode.

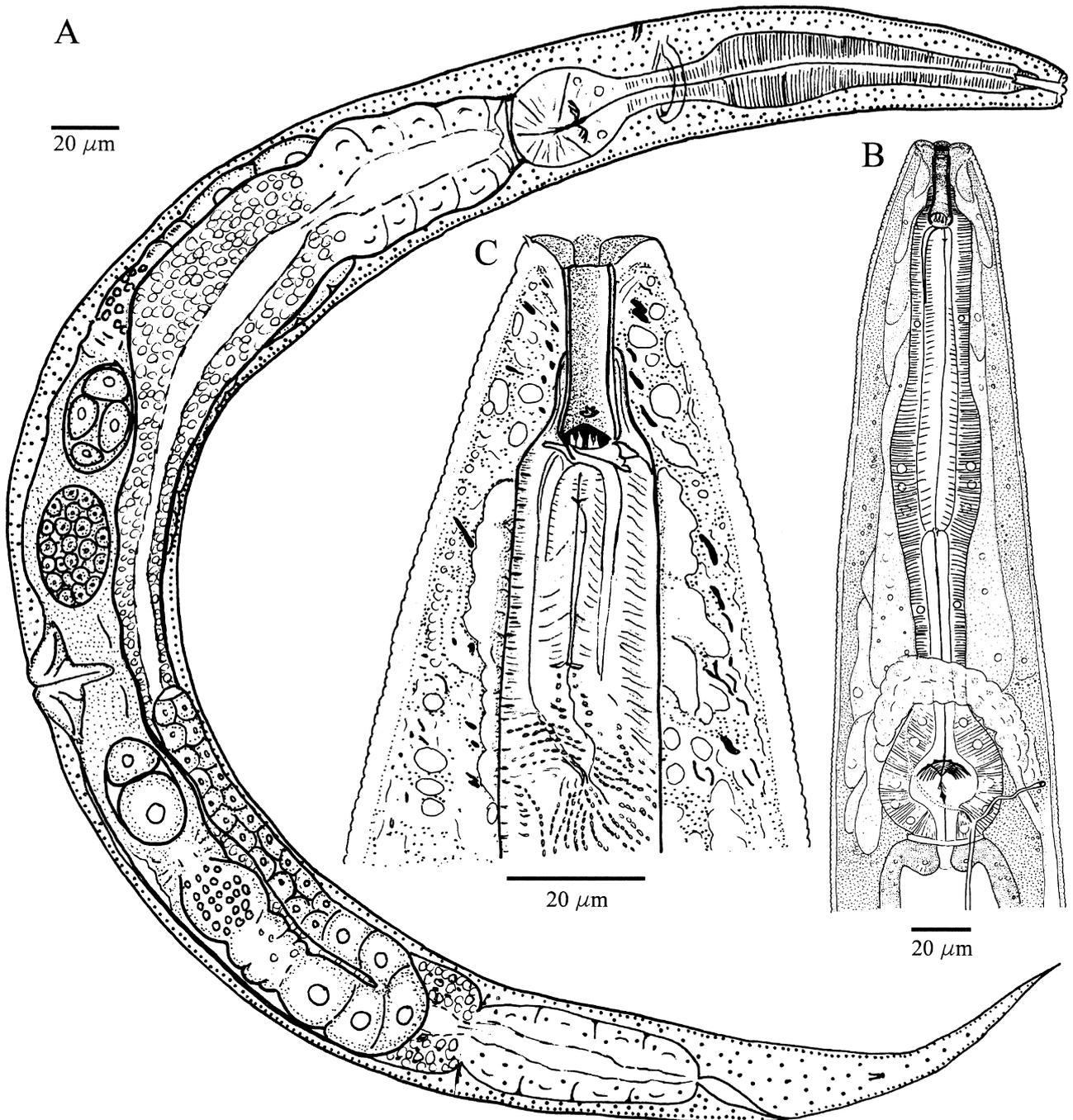


Fig. 1. *Rhabditis rainai* n. sp. Hermaphrodite. A: Entire body, LKC20; B: Stoma and pharynx, lateral view LKC20; C: Stoma, ventral view, PS1191.

each having at least 16 interrupted longitudinal lines beginning at level of pharynx, ending anterior of tail. Vulval lips protruding in some gravid individuals. Anterior

gonad length from vulva to reflex at distal gonad arm, slightly longer than posterior gonad arm; dorsal arm extending 80-100% of ventral arm. Lengths of both gonad

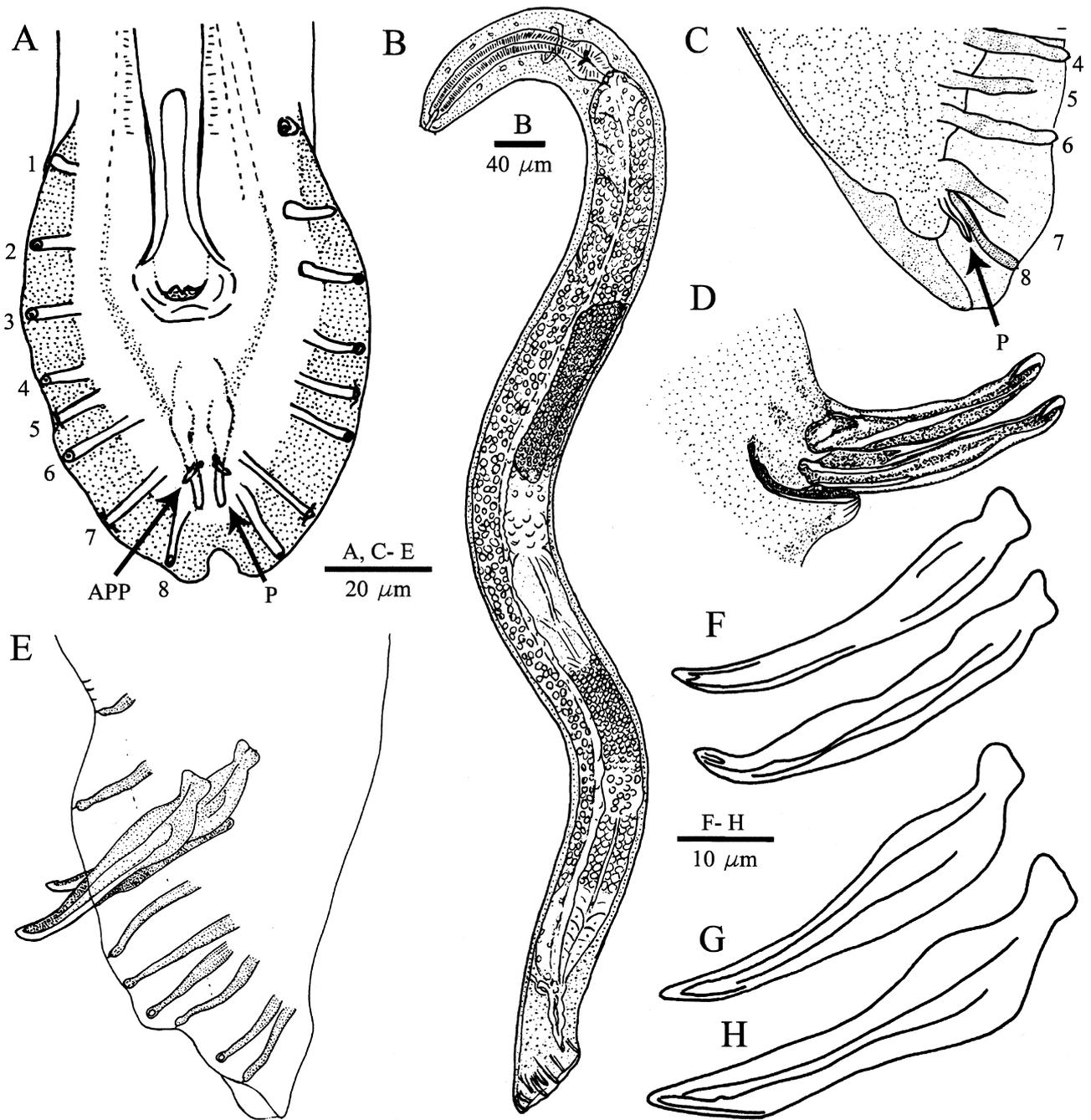


Fig. 2. *Rhabditis rainai* n. sp. Male. A: Tail, ventral view, showing rays, appendage (APP, arrow) and phasmid (P, arrow); B: Entire body; C: Tail, dorsolateral drawing of carmine-stained phasmid papilla bisected by stained central core (arrow), PS1191; D: Protruded spicules and gubernaculum; E: Lateral view of tail region showing spicules, gubernaculum, rays and bursa; F: LKC20 spicules, lateral view; G: *Rhabditis blumi* DF5010 spicule, lateral view; H: *Rhabditis adenobia* spicule, lateral view.

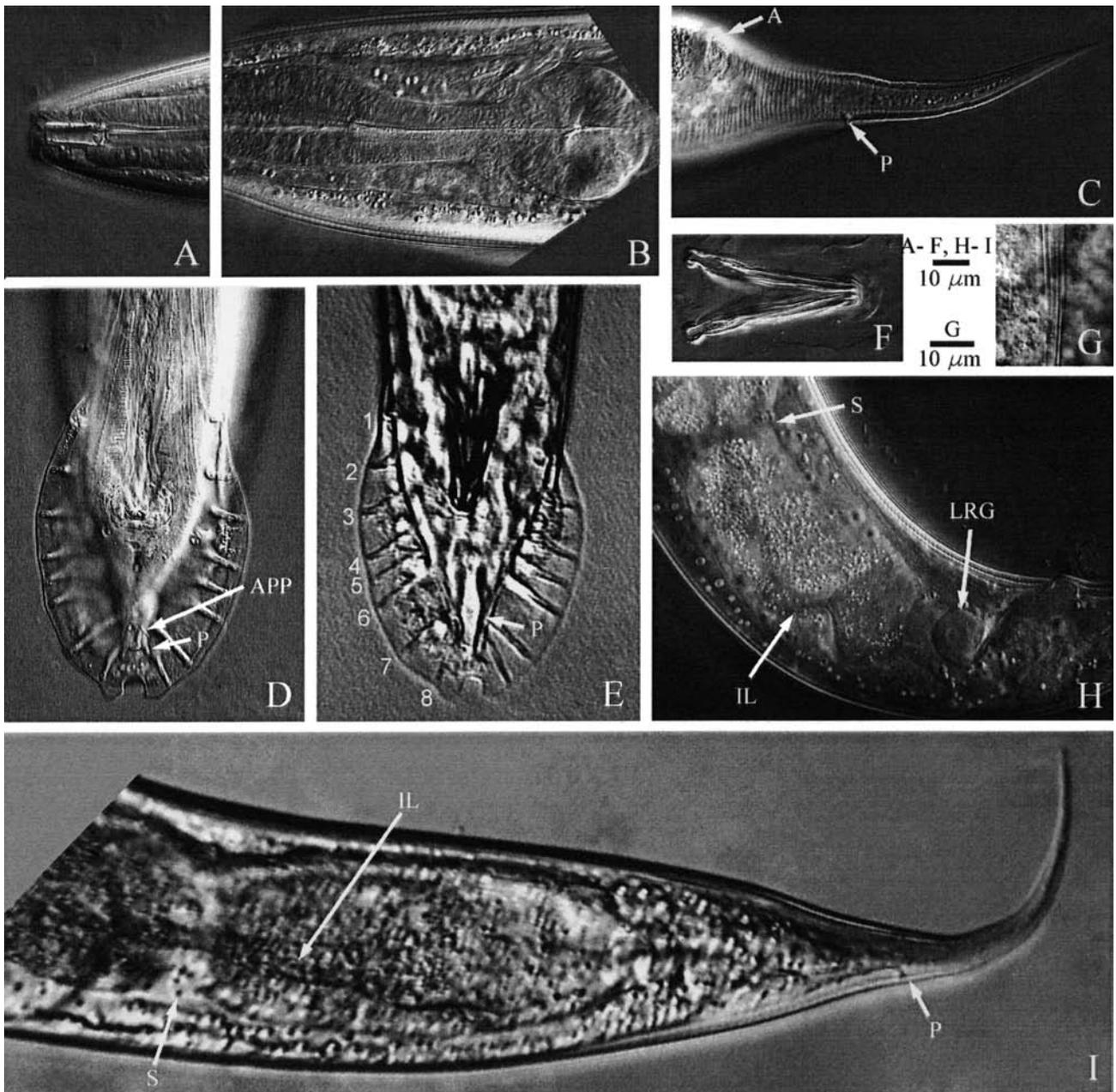


Fig. 3. *Rhabditis rainai* n. sp. Hermaphrodite. A: Stoma denticles and collar; B: Posterior pharynx; C: Tail, ventro-lateral view, with anus (A) and phasmid (P); G: Lateral field with six lines forming five ridges; H: Lateral view of contracted prerectum with stricture (S), intestinal lumen (IL), lateral rectal gland (LRG); I: Ventral view of extended prerectum, stricture (S), intestinal lumen (IL), phasmid (P). Male. D: Tail, superficial ventral view with peloderan bursa and notched tip, spicule distal tips, paired appendages (APP) and phasmid opening (P); E: Tail, interior ventral view, peloderan bursa with notch, spicule, phasmid canal (P) and sequentially numbered rays; F: Spicules, ventral view.

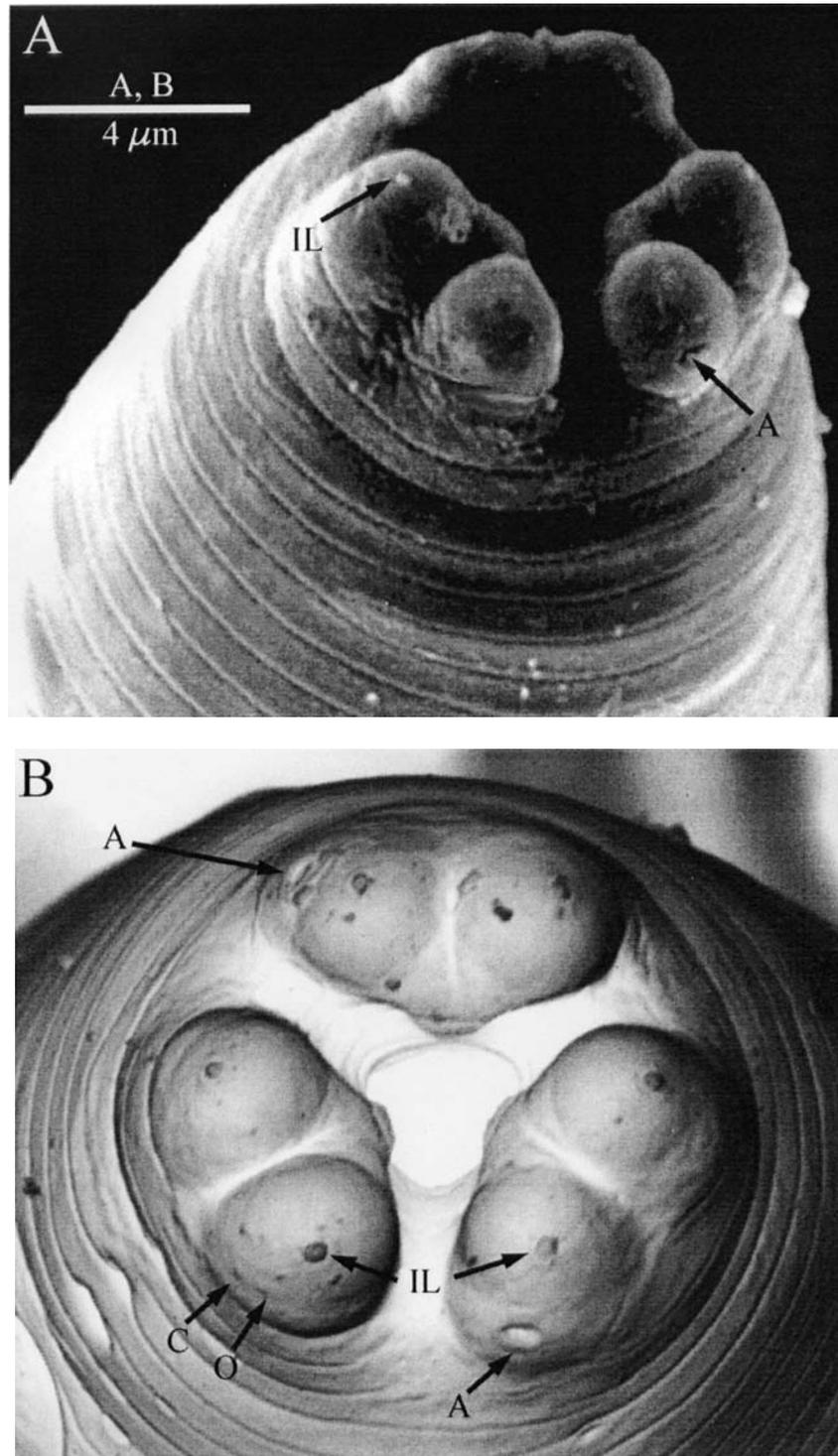


Fig. 4. *Rhabditis rainai* n. sp. Hermaphrodite, Scanning electron micrographs of anterior end. A: Oblique view (ventral to left); B: Face view (ventral to upper right). Abbreviations: arrow + A = amphid; arrow + C = cephalic sensilla; arrow + O = outer labial sensilla; arrow + IL = Inner labial sensilla. Assignments based on Riddle (1988) and Ward et al. (1975).

Table 1. *Rhabditis rainai* n. sp. Morphometrics of hermaphrodites from the type population (LKC20) and from population PS1191. All measurements are in μm and are in the form: mean \pm s.d. (range).

| Character | LKC20 (type population) | | | PS1191 (Fiji population) | |
|------------------------|-------------------------|-------------------------------|-------------------------------|-------------------------------|------------------------------|
| | fixed | | heat-killed | fixed | heat-killed |
| | Holotype | Paratypes | Paratypes | | |
| n | – | 20 | 15 | 20 | 30 |
| L | 1311 | 1120 \pm 161 (893-1587) | 1305 \pm 243 (941-1587) | 1269 \pm 84 (1093-1416) | 1245 \pm 233 (884-1748) |
| a | 12.5 | 14.5 \pm 1.8 (11.4-17.2) | 13.7 \pm 1.4 (10.1-15.8) | 14.3 \pm 1.2 (12.4-16.4) | 13 \pm 2 (10-17.7) |
| b | 6.6 | 6.2 \pm 0.6 (5.3-8) | 7.3 \pm 1.3 (5.2-9.3) | 7.8 \pm 0.7 (6.9-9.9) | 6.8 \pm 1.1 (5-9.4) |
| c | 10.2 | 10.7 \pm 1.4 (8.5-15.2) | 12.7 \pm 1.3 (10.1-14.4) | 12.2 \pm 1.5 (9.4-15.7) | 9.8 \pm 2.4 (6.6-19.3) |
| c' | 4 | 3.8 \pm 0.4 (3.2-4.7) | 2.8 \pm 0.25 (2.4-3.3) | 3.1 \pm 0.9 (2.2-3.9) | 3.9 \pm 0.7 (2.9-5.4) |
| V | 52 | 53 \pm 2 (50-59) | 52 \pm 2 (49-56) | 51 \pm 2 (46-55) | 51 \pm 2 (46-54) |
| Max body diam. | 105 | 79 \pm 17 (57-124) | 97 \pm 24 (67-143) | 92 \pm 12 (67-114) | 99 \pm 27 (56-149) |
| Pharynx | 200 | 181 \pm 12 (162-200) | 178 \pm 15 (152-200) | 163 \pm 10 (133-176) | 182 \pm 11 (163-205) |
| Tail | 128 | 105 \pm 12 (86-127) | 102 \pm 11 (86-114) | 105 \pm 11 (86-124) | 134 \pm 17 (90-166) |
| Rectum | 37 | 36 \pm 4 (27-46) | 38 \pm 3 (29-42) | 37 \pm 4 (27-42) | 40 \pm 8 (32-58) |
| Prerectum | 98 | 99 \pm 15 (79-140) | 109 \pm 19 (80-143) | 105 \pm 17 (85-163) | 116 \pm 21 (94-151) |
| Anal body diam. | 32 | 28 \pm 2.5 (24-32) | 37 \pm 4 (31-42) | 35 \pm 6 (29-57) | 36 \pm 6 (20-45) |
| Stoma length | 26 | 22 \pm 2 (19-26) | 22 \pm 2 (19-26) | 19.5 \pm 1 (18-20) | 24 \pm 3 (15-29) |
| Stoma diam. | 4 | 5 \pm 0.6 (4-6) | 4.6 \pm 0.6 (4-6) | 4.2 \pm 0.5 (3-5) | 4.6 \pm 0.8 (3-7) |
| Excretory pore to lips | 222 | 188 \pm 14 (163-222) | 201 \pm 30 (151-249) | 153 \pm 7 (153-197) | 197 \pm 35 (147-274) |
| Phasmid to anus | 41 | 36 \pm 6 (26-45) | 44 \pm 5 (38-54) | 34 \pm 3 (29-42) | 45 \pm 10 (26-74) |
| Anterior gonad | 380 | 312 \pm 69 (209-504) | 391 \pm 110 (209-551) | 384 \pm 43 (314-466) | 377 \pm 97 (190-580) |
| Posterior gonad | 333 | 279 \pm 67 (181-409) | 385 \pm 111 (209-542) | 387 \pm 46 (313-456) | 377 \pm 135 (247-665) |

arms representing 40-60% of body length. Uterus connected by sphincter to rounded spermatheca with sperm (2.2-6.5 μm diam.). Up to 11 embryos within uteri. Single row of oocytes at flexure of gonad arms. Distal ends of reflexed ovaries approaching level of vulva. Oval pseudo-coelomocytes present beyond both gonad flexures, 10-20 \times 15-27 μm in size. Posterior gonad arm flexure

mostly anterior to, never overlapping, anterior of prerectum. Postdeirid anterior to anterior prerectum where a constriction tethers intestine to body wall; prerectum *ca* three anal body diam. long, comprising five to six intestinal cells per side, ending at intestinal-rectal junction. Junction surrounded by three pear-shaped rectal sphincter cell bodies described as rectal glands. Rectum may be in-

Table 2. *Rhabditis rainai* n. sp. Morphometrics of males from the type population (LKC20) and from population PS1191. All measurements are in μm and are in the form: mean \pm s.d. (range).

| Character | LKC20 (type population) | | PS1191 (Fiji population) | |
|-----------------------------|-------------------------------|-------------------------------|-------------------------------|-----------------------------|
| | fixed | heat-killed | fixed | heat-killed |
| | Paratypes | Paratypes | | |
| n | 18 | 10 | 20 | 20 |
| L | 1001 \pm 83 (860-1169) | 910 \pm 123 (770-1140) | 739 \pm 37 (694-817) | 702 \pm 88 (627-878) |
| a | 16.8 \pm 2 (12.2-20.8) | 15.6 \pm 1.1 (13.3-17.2) | 15.9 \pm 1.6 (12.0-18.5) | 11.9 \pm 0.9 (10.4-14) |
| b | 6.2 \pm 0.5 (5.3-7.2) | 5.5 \pm 0.7 (4.2-6.7) | 5.4 \pm 0.4 (4.7-6.5) | 4.9 \pm 0.5 (4.4-6) |
| c | 21.1 \pm 1.9 (16.8-23.9) | 19.6 \pm 2.2 (17.3-23.5) | 22 \pm 1.6 (18-24) | 21.6 \pm 2.7 (18-28) |
| c' | 1.3 \pm 0.1 (1.1-1.5) | 1.2 \pm 0.1 (1.0-1.4) | 1.5 \pm 0.2 (1.0-1.7) | 1.2 \pm 0.1 (0.9-1.4) |
| Max. body diam. | 60 \pm 9 (48-76) | 59 \pm 11 (48-86) | 47 \pm 6 (38-67) | 59 \pm 7 (49-76) |
| Pharynx | 161 \pm 6 (152-171) | 166 \pm 17 (138-190) | 138 \pm 8 (119-150) | 144 \pm 4 (139-152) |
| Tail | 48 \pm 4 (40-54) | 47 \pm 6 (42-60) | 36 \pm 2 (29-38) | 33 \pm 3 (30-39) |
| Spicule length ¹ | 49 \pm 1.4 (46-52) | 43 \pm 4.0 (36-49) | 45 \pm 3.6 (41-59) | 38.8 \pm 2.2 (36-43) |
| Gubernaculum | 25 \pm 2 (22-29) | 23 \pm 3 (17-27) | 24 \pm 2 (20-28) | 19 \pm 2 (16-24) |
| Prerectum | 116 \pm 9 (99-128) | 112 \pm 17 (96-134) | 100 \pm 16 (77-139) | ² |
| Anal body diam. | 36 \pm 4 (28-43) | 38 \pm 6 (32-51) | 25 \pm 3 (23-38) | 27 \pm 3 (24-34) |
| Stoma length | 21 \pm 1.3 (19-23) | 21 \pm 3 (17-24) | 19 \pm 0.7 (18-20) | 16 \pm 0.9 (14-18) |
| Stoma diam. | 4.0 \pm 0.4 (3-5) | 3.9 \pm 0.4 (3-4) | 3.2 \pm 0.5 (3-4) | 3.1 \pm 0.4 (2.2-4) |
| Excretory pore to lips | 186 \pm 13 (167-214) | 179 \pm 17 (156-210) | 141 \pm 10 (122-161) | 113 \pm 7 (99-130) |
| Testis length | 618 \pm 60 (532-713) | 565 \pm 121 (435-788) | 389 \pm 48 (302-485) | 587 \pm 75 (527-796) |
| Testis reflexion | 140 \pm 22 (86-181) | 128 \pm 26 (101-179) | 89 \pm 14 (60-110) | ⁻² |

¹ Spicule length from head to tip, outside blade axis, 4(2-8)% shorter than actual axis length.

² Prerectum was not recognised nor was a testis reflex length measured in 1995. A few males were generated from starved plates in later years, but these could not be maintained to produce more progeny for new measurements.

Table 3. *Rhabditis rainai* n. sp. Morphometrics of dauer juveniles from the type population (LKC20) and from population PS1191. All measurements are in μm and are in the form: mean \pm s.d. (range).

| Character | LKC20 (type population) | | PS1191 (Fiji population) | |
|------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | fixed | heat-killed | fixed | heat-killed |
| | Paratypes | Paratypes | | |
| n | 10 | 10 | 6 | 9 |
| L | 369 \pm 59 (307-512) | 446 \pm 60 (337-523) | 467 \pm 71 (371-561) | 451 \pm 56 (344-530) |
| a | 18.7 \pm 1.1 (16.5-20.5) | 20.9 \pm 2.5 (14.7-23.7) | 17.2 \pm 0.6 (16.3-17.7) | 17.5 \pm 1.9 (14.8-19.2) |
| b | 3.4 \pm 0.5 (3-4.6) | 3.8 \pm 0.6 (2.6-4.5) | 3.9 \pm 0.6 (3.3-4.8) | 3.9 \pm 0.5 (3.1-4.5) |
| c | 8.0 \pm 0.4 (7.7-8.6) | 8.9 \pm 2.2 (5.5-11.9) | 9.0 \pm 1.8 (6.8-11.6) | 9.3 \pm 2.3 (6.5-14.1) |
| c' | 3.5 \pm 0.25 (3.1-3.8) | 3.8 \pm 1 (2.4-4.9) | 3.6 \pm 0.2 (3.4-3.9) | 3.5 \pm 0.4 (2.7-3.9) |
| Max. body diam. | 20 \pm 3 (19-28) | 22 \pm 3 (17-27) | 27 \pm 4 (21-33) | 25 \pm 3 (20-28) |
| Pharynx | 108 \pm 5 (102-112) | 119 \pm 9 (102-134) | 120 \pm 6 (112-129) | 115 \pm 9 (103-124) |
| Tail | 46 \pm 6 (40-60) | 52 \pm 10 (41-70) | 52 \pm 5 (45-57) | 50 \pm 8 (30-68) |
| Gonad primordium length | 31 \pm 5 (23-37) | 29 \pm 5 (19-34) | 25 \pm 1 (n = 4) | 25 \pm 1 (n = 4) |
| Mid-primordium /L (%) ¹ | 57 \pm 2 (55-61) | 55 \pm 2 (52-58) | 55 \pm 2 (n = 4) | 60 \pm 7 (n = 4) |
| Rectum | 16 \pm 4 (12-24) | 17 \pm 2 (14-19) | 13.6 \pm 1.5 (13-15) | 14.7 \pm 2.2 (13-18) |
| Prerectum | 39 \pm 3 (35-45) | 39 \pm 3 (36-41) | 46 \pm 2 (45-49) | 42 \pm 10 (26-49) |
| Anal body diam. | 13 \pm 2 (12-17) | 14 \pm 3 (11-18) | 15 \pm 1 (13-17) | 14 \pm 3 (9-18) |
| Stoma length | 19 \pm 1 (17-20) | 19 \pm 2 (17-21) | 19 (19) | 20 \pm 1 (19-22) |
| Stoma diam. | 2 \pm 0.5 (1-3) | 2.2 \pm 0.3 (1.7-2.6) | 2.6 \pm 0 (2.6-2.6) | 2.4 \pm 0.3 (2-3) |
| Excretory pore to lips | 91 \pm 5 (86-99) | 105 \pm 5 (97-114) | 121 \pm 7 (112-131) | 121 \pm 7 (118-127) |

¹ Distance from anterior end to middle of gonad primordium as % of body length.

flated, 1-1.6 anal body diam. long. Lower lip of anus variably protruding. Phasmid openings on papillae, 20-50% of tail from anus to tip. Conical, attenuated tail tapering smoothly.

Male

Stoma as for hermaphrodite. Faint lateral ridge present on cuticle at level of anterior isthmus, becoming three faint lines behind excretory pore level until distal testis level; then three faint ridges throughout midbody, ending as one near bursa. At least four interrupted cuticular longitudinal lines on dorsal and ventral sides next to lateral fields, ending with eight per side anterior to tail. Testis 38-73% of body length, with ventral reflex 15-30% of testis length. Prerectum present. Oval-shaped, open peloderan bursal velum with notch at tip with variable degree of indentation. Eight genital papillae (GP) or rays integrated into bursa. Ray pattern (1 + 2 + 3 + 2), first three pre-cloacal. Distance between rays 1 and 2 greater than between 2 and 3; distance between rays 3 and 4 slightly greater than between 4 and 5; distance between rays 6 and 8 twice distance between rays 5 and 6. Distal end of sequentially numbered rays 5 and 7 directed dorsally out of bursa, not extending to perimeter of bursa. Minute, very tiny paired papillar appendages visible between rays 7 and 8 in superficial ventral view, with slender canal of paired phasmids visible in deeper focal plane, lateral to papillar appendages; phasmid openings anterior to bursal notch, inside 8th pair of rays. Spicules separate with axis slightly curved, head apex flattened with slight bifurcation; constricted 'neck' or calomus region of shaft anterior to narrow shoulders; blade converging until the final tenth of its length with upward 35-40% curvature from blade axis; distal tip rounded, bearing ellipsoid, curved ventral arch. Spicule 36-59 μm long measured directly from head to tip, 2-8% shorter than the 37-61 μm distance along spicule axis. Gubernaculum in lateral view with medial fin-like expansion between half to two thirds distance from anterior end; minor expansion sometimes visible anterior to major one; in ventral view an ellipse with truncate ends. Three pear-shaped rectal glands located at intestinal-rectal junction.

Dauer juvenile

Ensheathed, with narrowed stoma, distally occluded. Nictation behaviour not observed. Prerectum not observed. Four lines in lateral field beginning at middle of procorpus, ending on tail.

Egg

Oval, egg shell hyaline without visible markings. LKC20 population (n = 20) length 60 ± 9 (47-80) μm ; diam. 37 ± 4 (29-45) μm . PS1191 population (n = 20) length 61 ± 3 (55-66) μm ; diam. 35 ± 3 (29-42) μm .

TYPE HOST AND LOCALITY

The type population, LKC20, was found in the gut and head of *Coptotermes formosanus* at the University of New Orleans, New Orleans, LA, USA.

OTHER LOCALITY

The second population, PS1191, was isolated from soil particles from Kioa Island, Fiji.

TYPE MATERIAL

Holotype (LKC20 hermaphrodite, ventro-lateral view in glycerin) from bacterial subculture on NGM agar with *E. coli* OP50, originally isolated from Formosan subterranean termite. Slide deposited in the USDANC, Beltsville, MD, USA. Paratypes (hermaphrodites, males) deposited in USDANC, Beltsville, MD, USA, population LKC20: 20 hermaphrodites, 18 males; population PS1191: 20 hermaphrodites, 20 males; Population LKC20 at other locations: the Florida Collection of Nematodes Gainesville, FL, USA (nine hermaphrodites, two males); the University of California, Riverside, Nematode Collection, Riverside, CA, USA (nine hermaphrodites, two males); Rothamsted Experimental Station Nematode Collection, Harpenden, Hertfordshire, UK (ten hermaphrodites, two males); the German National Collection, DNST, Münster, Germany (nine hermaphrodites, two males); and the Zoosystematical and the Ecological Institute, Eötvös Loránd University, Budapest, Hungary (ten hermaphrodites, two males).

DIAGNOSIS AND RELATIONSHIPS

Rhabditis rainai n. sp. is characterised by a hermaphroditic condition with males rare in nature, adults of each sex having a prerectum, papilliform phasmids, lip sectors fused in three pairs separated by grooves, and curved base of metastegosom wider than gymnostom, each glottoid swelling supporting four denticles. Hermaphrodites have a protruding posterior anal lip and the midbody lateral field has five ridges with three more lines sometimes visible. Males have three ridges in lateral field, bursa with posterior notch and eight rays; gently curved spicule with apically flattened, otherwise rounded head, constricted neck, narrow shoulders, final tenth of blade angled 35-40° from main axis with rounded distal tip supporting small ventral arch.

Rhabditis rainai n. sp. differs from species in the *Rhabditis* (*Oscheius*) *dolichura*-group (Sudhaus & Hooper,

Table 4. Comparison of morphometrics of female *Rhabditis* species. All measurements are in μm and, when cited in full, are in the form: mean \pm s.d. (range).

| Species | <i>R. adenobia</i> Poinar, 1971 | | <i>R. blumi</i> Sudhaus, 1974 | | <i>R. blumi</i> DF5010 heat-killed | <i>R. silvatica</i> Volz, 1951 | <i>R. rainai</i> n. sp. LKC20, PS1191, fixed, heat-killed |
|-------------------------------------|---|------------------------------|----------------------------------|-----------------------------|--|-----------------------------------|--|
| Source | 1971 | 5-11-70 ¹ | 1974 | 2-9-83 ² | 2005 | 1951 | 2005 |
| n | 15 | 4 | 10 | 4 | 10 | 13 ³ | 85 |
| L | 1200 (1056-1296) | 1264 \pm 30 (1226-1292) | – (1324-1819) | 1017 \pm 61 (912-1064) | 1381 \pm 292 (1102-1758) | – (746-1156) | – (884-1748) |
| a | – (18.8-22.9) | – | – (18-23) | 26.4 \pm 1.5 (24-27.5) | 15.9 \pm 2.8 (11-20) | – (15.5-19.5) | – (10-17.7) |
| b | – (5.1-5.4) | – | – (5-6) | 4.1 \pm 0.04 (4.1-4.2) | 6.1 \pm 0.8 (5.3-7.2) | – (4.5-5.6) | – (5-9.9) |
| c | – (11.3-11.8) | – | – (7-10) | 7.6 \pm 2.3 (5.7-11.1) | 11.1 \pm 2.2 (7.7-14.8) | – (5.8-8.6) | – (6.6-19.3) |
| c' | – | 3.7 \pm 0.3 (3.5-4.1) | – | 5.5 (4.4-8.9) | 6.5 \pm 1.8 (3.0-4.7) | 5.3 – | – (2.2-5.4) |
| V | 53 (49-56) | – | – (48-52) | – | 53 (49-57) | – (47-51) | – (46-56) |
| Max. body diam. | 55 (46-69) | 61 \pm 6 (52-67) | – (59-85) | 39 \pm 1 (38-41) | 92 \pm 34 (57-152) | – (47-72) | – (56-149) |
| Pharynx | 223 (208-239) | 223 \pm 18 (200-238) | – (244-298) | 246 \pm 16 (219-257) | 224 \pm 26 (181-266) | – (148-183) | – (133-205) |
| Tail | 102 (93-115) | – | – (156-221) | 142 \pm 35 (96-175) | 129 \pm 39 (89-221) | – (126-153) | – (86-166) |
| Anal body diam. | 29 (25-31) | – | 41.7 – | 22 \pm 3 (18-24) | 31 \pm 6 (24-40) | 29 | – (21-45) |
| Stoma ⁴ | 23 (20-27) (derived lips + stoma) | – | – (29-35) | 23.4 \pm 2.5 (20-27) | 29 \pm 5 (22-36) | 24 | – (15-29) |
| Stoma diam. | – | 4.5 \pm 0.9 (3.8-5.1) | – (3-5) | 3.6 \pm 0.6 (3-5) | 5.1 \pm 0.7 (3.8-5.7) | 5 | – (2.5-7) |
| Collar length | 6 (5-8) | – | – | – | 14 (11-17) | 12 | 10 (8-14) |
| Collar as % of buccal cavity length | 28-35 | – | 54-63 | – | 47-59 | 50 | 46-77 |
| Collar as % of stoma length | – | – | 50-58 | – | 43-52 | 34 | 35-61 (n = 20) |
| Teeth per metarhabdion | 4-5 | – | 4 | – | 4 | 3 | 4 |
| Head to excretory pore | 175 (177-211) | – | – | – | – (208-227) n = 3 | 158 | – (145-275) |
| Excretory pore as % of pharynx | – | (84-98) | (72-86) | – | (91-114) | – | (81-152) |
| Anterior gonad length | 369 (267-423) | – | 174 \pm 28 (134-194) | – | 440 \pm 187 (219-741) | 278 | (205-567) |
| Posterior gonad length | 377 (285-447) | – | 194 \pm 43 (130-223) | – | 428 \pm 172 (238-741) | 265 | (177-651) |
| Prerectum | – | – | – | – | – | 60 | (77-140) |

Table 4. (Continued).

| Species | <i>R. adenobia</i> Poinar, 1971 | | <i>R. blumi</i> Sudhaus, 1974 | | <i>R. blumi</i> DF5010 heat-killed | <i>R. silvatica</i> Volz, 1951 | <i>R. rainai</i> n. sp. LKC20, PS1191, fixed, heat-killed |
|--------------------------------------|------------------------------------|----------------------|----------------------------------|---------------------|--|-----------------------------------|--|
| Source | 1971 | 5-11-70 ¹ | 1974 | 2-9-83 ² | 2005 | 1951 | 2005 |
| Rectum as % of tail length | 24 | – | – | – | 25-40 | 29 | 29-44 |
| Phasmid position as % of tail length | – | – | 25-31 | – | 26-41 | 35 | 29-38 |

¹ 5-11-70 slide T 847p: four females, Poinar (1971) new measurements for this comparison.

² 2-9-83 slide T 3064p: five agar culture females, Sudhaus (1974) new measurements for this comparison.

³ 1995 Single values in the column of *R. silvatica* were derived from original drawings.

⁴ Stoma L (buccal cavity, Bc, plus lip height) according to information in De Ley *et al.* (1995). Stoma L for *R. adenobia* derived from “length stoma” (= buccal cavity or stegostom plus gymnostom) plus “length free portion of lips” in Poinar (1971).

1994) which have nine bursal rays and spicules with swollen, uncurved distal tip without a ventral arch and minute, rather than elongated, stomatal teeth without an enlarged posterior stoma. *Rhabditis rainai* n. sp. differs from leptoderan-tailed, nine-ray species in the *Rhabditis* (*Oscheius*) *insectivora*-group (Sudhaus & Hooper, 1994) in having a peloderan tail with eight rays, expansile rectum and spicule lacking a distal hook. *Rhabditis silvatica* has a prerectum, but differs from *R. rainai* n. sp. in having a median pharyngeal bulb, three stomatal teeth, nine bursal rays with leptoderan male tail, lower ‘b’ ratio (4.5-5.6 vs 5-9.9) (Table 4), and smaller spicule axis length (34-38 vs 38-64 μm).

Rhabditis rainai n. sp. differs from *Rhabditis* (*Rhabditis*) *blumi* Sudhaus, 1974 and from *Rhabditis* (*Rhabditis*) *adenobia* Poinar, 1971 (Sudhaus, 1974) in the hermaphroditic condition with rare males induced by starvation, a peloderan rather than leptoderan tail, spicule not sabre-like with gentle neck curvature, wide shoulders, or with a blade curvature at 15-20% of length, but with less acute spicule tips bearing a unique ventral arch.

Compared to *R. (R.) adenobia* (Poinar, 1971), *R. rainai* n. sp. has a wider hermaphrodite body ($P < 0.001$ unless otherwise noted) (LKC20: 79, PS1191: 92 μm , range (56-149) μm vs *R. adenobia* 1971, 55 (46-69) μm ; or 2005, 61 (52-67) μm), and lower hermaphrodite a value (LKC20: 14.5 (11-17), PS1191: 14.3 (12-16) vs *R. adenobia* 20.8 (19-24)). *Rhabditis rainai* n. sp. LKC20 males have a longer body length than *R. adenobia* (1001 (860-1169) μm vs 926 (768-1248) μm , $P < 0.01$), while population PS1191 males have a shorter male body length (739 (694-817) μm), and shorter stoma (19 (18-20) μm vs 22 (20-24) μm , $P < 0.001$). *Rhabditis rainai* n. sp.

has a shorter stoma collar (6 (5-8) vs 10 (8-14) μm) and longitudinal cuticular markings unlike the amorphous cuticular pattern seen in fixed *R. adenobia*.

Compared to *R. blumi* (Sudhaus, 1974) *R. rainai* n. sp. has a smaller pharynx length [Hermaphrodite – fixed LKC20: 181 (162-200) μm or PS1191: 163 (133-176) μm vs *R. blumi* 1983: 246 (244-298) μm ; heat-killed LKC20: 178 (152-200) μm or PS1191: 182 (163-205) μm vs DF5010 2005: 224 (181-266) μm ; Male – fixed LKC20: 161 (152-171) μm , PS1191: 138 (119-150) μm vs *R. blumi* 1974: (226-276) μm , or heat-killed LKC20: 166 (138-190) μm , and PS1191: 144 (139-152) μm vs DF5010 2005: 191 (162-223) μm ; *R. blumi* pharynx fixed > heat-killed, $P < 0.05$); smaller hermaphrodite a value (fixed LKC20: 14.5 (11-17) μm , PS1191 14.3 (12-16) vs *R. blumi* 1983: 26.4 (24-28) μm , with discretely different ranges (10-17.7) vs (18-23) from *R. blumi* 1974 original values, but *R. rainai* heat-killed not different from heat-killed DF5010: 16 (11-20 μm)), somewhat lower hermaphrodite c value (*R. rainai* all populations (2.2-5.4) vs *R. blumi* 1974: (5.5) or 1983: (4.4-8.9), and only heat-killed LKC20: 2.8 (2.4-3.3) vs DF5010: 3.7 (3-4.7)), shorter spicule axis length (heat-killed LKC20: 45 (38-51) μm $P < 0.005$, PS1191: 41 (38-45) μm vs DF5010: 54 (48-63) μm ; fixed only LKC20: 51 (48-54) μm vs range of 1974 and 1983 fixed specimens (41-51) μm using median 46 μm), and markedly shorter testis reflex expressed as a percentage of testis length (20-28 vs 42-65%).

Qualitative features also differed between live cultures of *R. blumi* and *R. rainai* n. sp. Even well-fed individuals of *R. rainai* n. sp. had remarkably clear bodies with very few refractive globules of different shape, size and

Table 5. Comparison of morphometrics of male Rhabditis. All measurements are in μm and are, when cited in full, in the form: mean \pm s.d. (range).

| Species | <i>R. adenobia</i> Poinar, 1971 | | <i>R. blumi</i> Sudhaus, 1974 | | <i>R. blumi</i> DF5010 | <i>R. silvatica</i> Volz, 1951 | <i>R. rainai</i> n. sp. LKC20, PS1191, fixed, heat-killed |
|---------------------------------|------------------------------------|---------------------------|----------------------------------|----------------------------|-----------------------------|--------------------------------------|---|
| | fixed | heat-killed | fixed | heat-killed | heat-killed | 1951 | heat-killed |
| Source | 1971 | 1970-2005 ¹ | 1983-2005 ² | | 2005 | 1951 | 2005 |
| n | 15 | 4 | 10 | 5 | 7 | 7 | 62 |
| L | 926 (768-1248) | – | – (995-1415) | – | 996 \pm 212 (646-1264) | – (523-808) | – (627-1169) |
| a | – (16-25) | – | – (17-22) | – | 16 \pm 3 (12-20) | – (14-19) | – (10-21) |
| b | – (4.3-5.6) | – | – (4-6) | – | 5 \pm 1 (3-6) | – (4-5) | – (4-7) |
| c | – (19-24) | – | – (17-27) | – | 17 \pm 4 (12-24) | – (11-15) | – (20-28) |
| Max. body diam. | 45 (31-77) | – | – (49-68) | – | 66 \pm 18 (43-86) | – (35-44) | – (38-86) |
| Pharynx | 203 (177-233) | – | – (226-276) | – | 191 \pm 22 (162-223) | – (99-132) | – (119-190) |
| Tail | 46 (40-52) | – | – (50-66) | – | 59 \pm 10 (46-73) | – (40-52) | – (29-60) |
| Stoma ³ | 21 (19-25) | 22 \pm 2 (20-24) | – (25-27) | – | 24 \pm 2 (22-26) | 22 | – (14-24) |
| Stoma diam. | – | 4 \pm 0.6 (4-5) | – | 4.3 \pm 0.5 (4-5) | 4 \pm 1 (3-5) | 5 | – (2.2-5) |
| Collar length | 6 (4-9) | – | – | – | 11 \pm 2 (9-11) | 13 | 10 \pm 1 (9-13) |
| Collar as % of stoma length | – | – | 54-63 | – | – | 61 | 53-71 (n = 18) |
| Excretory pore | 173 (150-205) | – | – | 182 \pm 10 (168-191) | 201 \pm 22 (160-223) | – | – (99-214) |
| Spicule axis length | 47 (40-53) | – | – (45-51) | 43 \pm 2 (41-45) | 54 \pm 5 (48-63) | – (34-38) | – (38-64) |
| Gubernaculum | 21 (20-26) | – | – (16-22) | – | 27 \pm 6 (20-31) | – (16-19) | – (16-29) |
| Testis length | – | 520 \pm 22 (504-551) | – | 432 \pm 37 (385-470) | 753 \pm 150 (587-989) | 505 | – (302-796) |
| Testis reflexion | – | 137 \pm 5 (133-143) | – | 124 \pm 8.5 (111-134) | 388 \pm 66 (293-443) | 90 | – (60-181) |
| Reflexion length as % of testis | – | 26 (23-28) | – | 29 (26-31) | 51 (42-65) | 18 | 23 (20-28) |
| Tail shape | Leptoderan, notched | – | Leptoderan, notched | – | Leptoderan, notched | Leptoderan, unnotched | Peloderan, notched |

¹ 5-11-70 slide T 847p: four males from Poinar (1971) indicated here *X \pm SD, (range) indicate new measurements.

² 2-9-83 slide T 6039p: five males from Sudhaus (1974) represent new measurements.

³ Stoma L (buccal cavity plus lip length) according to information in De Ley *et al.* (1995); stoma L for *R. adenobia* derived from “length stoma” (= buccal cavity or stegostom plus gymnostom) plus “length free portion of lips” in Poinar (1971).

distribution; the cuticle was very coarsely annulated and tessellate. The stoma had more prominent circular striae

and the excretory pore was consistently anterior to the pharyngeal-intestinal junction, the unusually convoluted

excretory canal having to be traced through a number of focal planes. In *R. blumi* the intestine was extremely narrow and the posterior gonad at the curve of the reflex made a loop around and under itself and then around the intestine. In contrast, the *R. rainai* n. sp. gonad severely deformed the otherwise broad intestine in the midbody region and there was a simple fold in a single plane of the gonad reflex similar to that in *R. adenobia*. The gonad flexures extended closer to the anus and pharyngeal-intestinal junctions in *R. blumi* than in *R. rainai* n. sp. Observations of heat-inactivated *R. blumi* females, plus active females with intestinal movement, showed there was no constriction of the posterior intestinal lumen nor any tethering fibres from the body walls. However, in *R. blumi* males the *vas deferens* had an unusually large lumen that had a similar appearance to a prerectum in *R. rainai* n. sp.

The somewhat similar morphological features of *Rhabditis blumi* and *R. rainai* n. sp. are supported by some molecular characters from three genes (Kiontke & Fitch, 2005) that showed *Rhabditis* PS1191 in a clade with *Rhabditis blumi* having 100% bootstrap support. In this tree, *R. rainai* n. sp. PS 1191 was distantly related to the leptoderan *Rhabditis* (*Oscheius*) *insectivora*-group (Sudhaus & Hooper, 1994).

Remarks

HERMAPHRODITES

While both populations LKC20 and PS1191 had overlapping morphometric measurements, some significant variation ($P < 0.001$, unless otherwise noted) was noted among population averages. This occurred in surprising patterns both between the two geographic populations and within each population for live, heat-inactivated, or for worms fixed and processed to glycerin. *Rhabditis rainai* n. sp. PS1191 hermaphrodites had a significantly longer average tail length in heat-killed individuals (134 vs 102 μm) and a larger c' ratio (3.9 vs 2.8). Fixation-associated differences between PS1191 compared to LKC20 ($n = 20$, PS1191 vs LKC20) were noted in larger values for body length (1268 vs 1120 μm), body diam. (92 vs 79 μm), and b ratio (7.8 vs 6.2), and smaller values for stoma diam. (4.2 vs 5 μm), pharynx length (163 vs 181 μm), and excretory pore to lip distance (153 vs 188 μm). Significant differences in measurements associated with fixation occurred within population LKC20 (fixed vs heat-killed) for mid-body diam. (79 vs 97 μm),

anal body diam. (28 vs 37 μm), anterior gonad (312 vs 391 μm , $P < 0.01$), and posterior gonad (279 vs 385 μm , $P < 0.005$). In contrast, different length measurements from population PS1191 showed significant differences in fixed compared to heat-inactivated specimens for stoma length (19.5 vs 24 μm), pharynx length (163 vs 182 μm), excretory pore to lip length (153 vs 197 μm), prerectum length (105 vs 116 μm) and tail length (105 vs 134 μm).

MALES

Staining within the canal of the male phasmid papillae was observed as shown in Figure 2C. The small appendages on the male tail are in the expected position of the missing rays and may be vestigial. Heat-inactivated males of PS1191 compared to LKC20 had significantly ($P < 0.001$ unless otherwise noted) shorter values for stoma length (16 vs 21 μm), body length (702 vs 910 μm), pharynx length (144 vs 166 μm), excretory pore position (113 vs 179 μm), tail length (33 vs 47 μm), spicule (38.8 vs 43 μm , $P < 0.05$) and gubernaculum (19 vs 23 μm), and narrower stoma diam. (3.1 vs 3.9 μm), anal body diam. (27 vs 38 μm), and lower ratios a (11.9 vs 15.6) and b (4.9 vs 5.5, $P < 0.025$). Fixation-associated differences between PS1191 compared to LKC20 (PS1191 vs LKC20) were noted in smaller values for prerectum length (100 vs 116 μm) and testis length (389 vs 618 μm). Significant differences in measurements associated with fixation occurred within population LKC20 (fixed vs heat-killed) of mid-body diam. (79 vs 97 μm), anal body diam. (28 vs 37 μm), anterior gonad (312 vs 391 μm , $P = 0.01$) and posterior gonad (279 vs 385 μm , $P = 0.005$). In contrast, other length measurements from population PS1191 were significantly smaller in fixed compared to freshly heat-killed specimens for stoma length (19.5 vs 24 μm), pharynx length (163 vs 182 μm), excretory pore to lip distance (153 vs 197 μm), prerectum length (105 vs 116 μm) and tail length (105 vs 134 μm).

DAUER JUVENILES

Comparison of heat-killed population PS1191 with LKC20 demonstrated larger body diameter (heat-killed: 25 vs 22 μm , $P < 0.025$; fixed: 27 vs 20 μm , $P = 0.001$), smaller a value (17.5 vs 20.9, $P < 0.005$), longer head to excretory pore distance (heat-killed: 121 vs 105 μm , $P < 0.001$; fixed: 121 vs 91 μm), and shorter rectum (14.7 vs 17 μm , $P < 0.025$). A longer tail was noted (52 vs 46 μm , $P = 0.025$) only in fixed specimens.

A fixation-associated difference was only noted in LKC20 pharynx length (108 vs 119 μm , $P < 0.005$).

ECOLOGY

In the initial sample of nematode-infested termites, 90% lacked the flagellate symbionts necessary for cellulose digestion while all termite workers had juvenile and/or adult nematodes within their guts. No more than two nematodes were associated with each gut from an initial sample of sluggish termites with reduced grooming behaviour. However, in later samples collected the same month, up to nine nematodes were found in a single gut with a few being sometimes found in the head.

BIONOMICS

Rhabditis rainai n. sp. LKC20 generation time was 3 days at 25°C and 4 days at 20°C. Average live progeny brood size for LKC20 ($n = 11$) was 108 ± 9.4 (58-150), and for PS1191 ($n = 9$) was 199 ± 15 (41-216), which was significantly larger than LKC20 ($P < 0.001$). A small number of non-viable eggs were also laid.

Within 2 days of the mating of LKC20 males with PS1191 hermaphrodites, numerous young males were detected on both plates that could be distinguished from hermaphrodites by the characteristic tail swelling, narrower body and relatively rapid movement. These were determined to be cross-progeny of both populations since their numbers were much greater than expected compared to intentionally starved plates used to generate males at levels $< 0.1\%$. Male cross-progeny were fertile since subsequent F1 crosses resulted in F2 male progeny. A few PS1191 males were generated from starved plates, but these could not be maintained to produce more progeny for the reciprocal mating of PS1191 males and LKC20 hermaphrodites.

Discussion

The presence of a prerectum in *R. rainai* n. sp. is unusual for a rhabditid nematode, the other known exception being *Rhabditis silvatica* Volz, 1951 (Zell, 1983). The prerectum is characterised by an intestinal constriction some distance behind the rectal sphincter (Caviness, 1964), where gut contents are isolated before expulsion (Seymour & Doncaster, 1972; Bird & Bird, 1991). Since many taxonomic descriptions do not include information on the intestine, it is possible that a prerectum may have been missed in taxa where such a

character is usually uncommon. The structure is common in terrestrial adenophoreans (Maggenti, 1981), including Dorylaimida (Ji Hua & Yan Ling, 1999), Lep-tonchida (Dhanam & Jairajpuri, 1999) and Chromadorida (Tahseen, 2000). However, the prerectum has been observed in only a few other secernentean nematodes including *Diplenteron potohikus* Yeates, 1969 (Rhabditida: Neodiplogastridae) (Yeates, 1969a, b), *Aphelenchoides blastophthorus* (Aphelenchida) (Seymour & Doncaster, 1972), *Metacrobeles tessellatus* De Ley, Coomans & Geraert, 1990, *Deficephalobus desenderi* De Ley & Coomans, 1990 and, occasionally, in *Pseudacrobeles macrocystis* De Ley & Siddiqi, 1991 (Rhabditida: Cephalobidae). In the cephalobids it was associated with apparently modified pseudocoelomocytes (De Ley & Siddiqi, 1991).

Notable morphometric variability was demonstrated in this study. Understanding these patterns can be especially useful for accurate diagnoses when measurements from only live or fixed material are available for comparison. While fixation and sampling may partially account for some morphometric differences among *R. blumi* samples (Tables 4, 5) for live (1974, 2005) and fixed specimens (1983 slide), other factors are also likely. The *R. blumi* DF5010 population from the CGC (Sudhaus laboratory culture SB140), originally from the South of Valencia, Spain, was first cultured in April 1971 on agar with raw meat or potato. The change in nutritional media to a sparse regimen of *E. coli* on NGM may have influenced some morphometric differences, especially in female body length/diameter in the a ratio (Table 4). The long time in continuous laboratory culture could also have provided opportunity for mutations to accumulate. Many *C. elegans* laboratories request new worm cultures from frozen stock at the CGC every few years to avoid mutational load over time. While most of the qualitative features remained the same for *R. blumi*, the flagellum was no longer present at the end of the *R. blumi* tail after culture on *E. coli*. In one *R. blumi* culture from the CGC that had been continuously subcultured for nearly 2 years, many tails had shorter, lumpy, profiles, including short stubs similar to the unusual 'knotted tails' detected in various other species of *Rhabditis* (Osche, 1954; Sudhaus, 1976). A second CGC culture received in March 2005 had a uniformly tapering tail, but was also shorter, lacking the long flagellum typical of early specimens. The shortened tails account for the differences in the c ratio between the 2005 live culture (7.7-14.8, shortest tail 89 μm), 1983 slide (5.7-11.1, shortest tail 96 μm) and 1974 (7-10, shortest tail 156 μm) measurements (Table 4). In contrast

to this pattern, *R. rainai* n. sp. PS1191 was in culture for approximately 10 years longer than LKC20, and still exhibited a longer, 'unknotted', tail. However, generating males from PS1191 was more difficult recently compared to 10 years previously. This difficulty associated with older cultures has also been noted in other species of Rhabditida (Hooper *et al.*, 1999).

The populations of *R. rainai* n. sp. and related species of *R. blumi* and *R. adenobia* studied herein do not fit well into existing genera (Andrássy, 1984) or subgenera (Sudhaus & Fitch, 2001) within the Rhabditidae, especially within *Rhabditis* (*Rhabditis*) where leptoderan taxa with eight and nine bursal rays now co-exist (Sudhaus & Fitch, 2001). When more taxa and characters can be presented, a new subgenus for *R. rainai* n. sp. might be considered appropriate.

Rhabditis rainai n. sp. was apparently detected, but not fully described, more than 18 years ago from the same termites and geographic region and had a similar generation time, brood size and habit within termites (Coppel & Liang, 1987). The simple drawing of the hermaphrodite in that report showed the absence of a median pharyngeal bulb and presence of an intestinal constriction consistent with the appearance of the rarely occurring pre-rectum. While many bacterial-feeding rhabditid nematodes are phoretic associates of insects that are generally acquired in the dauer juvenile form (Poinar, 1975), the *R. rainai* n. sp. LKC20 type population was found with multiple non-dauer life stages, primarily in the gut and head of live termites. Different numbers and types of nematode life-stages in related insect hosts may reflect a range of benign to destructive roles for rhabditid nematode associates. *Rhabditis adenobia* could be cultured *in vitro*, but also lived and reproduced within the colleterial glands and male endophallic passages of the dynastid beetle *Oryctes monoceros* L. (Coleoptera: Scarabaeidae) without apparent harm to the host (Poinar, 1971). *Rhabditis* (*Oscheius*) *necromena* Sudhaus & Schulte, 1989 infested three species of millipede to different degrees. In its native Australian host *Akamptogonus novarae* (Humbert & Saussure) (Diplopoda: Paradoxosomatidae), *R. necromena* existed in large numbers in a necromenic relationship where a developmentally-arrested dauer juvenile inhabited a phoretic-host haemocoel before consuming bacteria reproducing in a naturally-occurring cadaver. *Rhabditis necromena* was also demonstrated to exist in dauer form, but in small numbers within Australian *Oncocladostoma castaneum* (Attems) (Diplopoda: Paradoxosomatidae). However, in the invasive Portuguese pest *Om-*

matoius moreletii (Lucas) (Diplopoda: Iulidae), a lethal infection occurred after ingestion of dauer juveniles from which saprotrophic stages grew in the body cavity along with lethal bacteria (Schulte, 1989). *Rhabditis rainai* n. sp. dauer larvae experimentally infested two other subterranean termite species native to the USA, *Reticulitermes flavipes* Kollar and *R. hesperus* Banks (Massey, 1971), occurring in these insects in multiple life-stages. No mention was made of any variability in infection among the three termites (Coppel & Liang, 1987). It would not be surprising if *R. rainai* n. sp. also existed as a more benign dauer form within some related insect in southeast Asia where the nematode may be endemic. This speculation is based on the type locality of *Coptotermes formosanus* from Taiwan (Grassé, 1984) and conspecific population PS1191 from Fiji. However, it is also possible that *R. rainai* n. sp. could be native to the USA as the nematode was found in *Reticulitermes virginicus* from Gulfport, MS, USA (Coppel & Liang, 1987), where it may or may not have encountered *C. formosanus* infested with *R. rainai* n. sp. If *R. rainai* n. sp. normally inhabited the termites in low numbers as dauer juveniles (daurers may have grown into saprophytes before they could be observed in these termites), the moist sprinkler site where infested termites were found would facilitate transmission not only of these desiccation-resistant dauer juveniles (Coppel & Liang, 1987), but perhaps even saprotrophic stages. In previous infections of *C. formosanus* from Baton Rouge, LA, USA, where up to 30% mortality was recorded, most saprophytic nematode stages were present in the head, prothorax, femora and proventriculus, with only a few present in the gut (Coppel & Liang, 1987). The fact that in this study so many *R. rainai* n. sp. were found in the gut could be a result of reduced grooming behaviour associated with the insecticide imidocloprid (Admire[®], Bayer CropScience LP, Research Triangle Park, NC, USA) to which these termites were exposed (unpublished observations), possibly allowing mouth entry. The relationship of the nematode in the insect could be described as phoretic, moderately pathogenic, and facultatively parasitic, since the nematode also fed saprophytically.

Acknowledgements

The authors thank A. Raina, USDA-ARS, Formosan Subterranean Termite Research Unit, Southern Regional Research Center, New Orleans, LA, USA, for logistical help. We also thank Sharon Ochs, Katrina Campbell and Carl Wiemann, Nematology Laboratory, Beltsville, MD,

USA, for technical support and David Carta with statistical programming. We also thank the *Caenorhabditis* Genetics Centre for *Rhabditis blumi* DF5010.

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

References

- ANDRÁSSY, I. (1983). *A taxonomic review of the suborder Rhabditina (Nematoda: Secernentia)*. Paris, France, ORSTOM, 241 pp.
- ANDRÁSSY, I. (1984). *Klasse Nematoda (Ordnungen Monhysterida, Desmoscolecida, Araeolaimida, Chromadorida, Rhabditida)*. Berlin, Germany, Gustav Fischer Verlag, 509 pp.
- BARGMANN, C.I. & AVERY, L. (1995). Laser killing of cells in *Caenorhabditis elegans*. In: Epstein, H.F. & Shakes, D.C. (Eds). *Methods in cell biology*, Vol. 48. *Caenorhabditis elegans: model biological analysis of an organism*. New York, NY, USA, Academic Press, pp. 225-250.
- BIRD, A.F. & BIRD, J. (1991). *The structure of nematodes*. New York, NY, USA, Academic Press, 316 pp.
- CAVENESS, F.E. (1964). *A glossary of nematological terms*. Ibadan, Nigeria, The Pacific Printers, 68 pp.
- COPPEL, H.C. & LIANG, M.C. (1987). Rhabditoid nematodes associated with subterranean termites. *Forestry Research Notes, Department of Forestry, University of Wisconsin*. No. 274, 5 pp.
- DE LEY, P. & SIDDIQI, M.R. (1991). *Pseudacrobeles macrocystis* sp. n., with some new observations on the morphology of Cephalobidae (Nematoda). *Afro-Asian Journal of Nematology* 1, 31-40.
- DE LEY, P., VAN DE VELDE, M.C., MOUNPORT, D., BAUJARD, P. & COOMANS, A. (1995). Ultrastructure of the stoma in Cephalobidae, Panagrolaimidae and Rhabditidae, with a proposal for a revised stoma terminology in Rhabditida (Nematoda) *Nematologica* 41, 153-182.
- DHANAM, M. & JAIRAJPURI, M.S. (1999). New leptonchid nematodes: one new genus and eleven new species from Malnad Tracts of Karnataka, India. *International Journal of Nematology* 9, 1-18.
- GOLDEN, A.M. (1990). Preparation and mounting nematodes for microscopic observation In: Zuckerman, B.M., Mai, W.F. & Krusberg, L.R. (Eds). *Plant nematology laboratory manual*. Revised Edition. Amherst, MA, USA, University of Massachusetts Agricultural Experimental Station, pp. 200-201.
- GRASSÉ, P.-P. (1984). *Termitologia. Tome II*. Paris, France, Masson & Cie, 613 pp.
- HOOPER, D.J., WILSON, M.J., ROWE, J.A. & GLEN, D.M. (1999). Some observations on the morphology and protein profiles of the slug-parasitic nematodes *Phasmarhabditis hermaphrodita* and *P. neopapillosa* (Nematoda: Rhabditidae). *Nematology* 1, 173-182.
- JI HUA, W. & YAN LING, L. (1999). Two new species of Actinolaimidae Thorne, 1939 (Nemata: Dorylaimida) from China. *Journal of Nematology* 31, 475-481.
- KIONTKE, K. & FITCH, D.H.A. (2005). The phylogenetic relationships of *Caenorhabditis* and other rhabditids. In: *WormBook*, The *C. elegans* research community (Ed.). <http://www.wormbook.org> (8-11-05).
- KIONTKE, K. & SUDHAUS, W. (2000). Phasmids in male Rhabditida and other secernentean nematodes. *Journal of Nematode Morphology and Systematics* 3, 1-37.
- MAGGENTI, A. (1981). *General nematology*. New York, NY, USA, Springer Verlag, 372 pp.
- MASSEY, C.L. (1971). Two new genera of nematodes parasitic in the eastern subterranean termites *Reticulitermes flavipes*. *Journal of Invertebrate Pathology* 17, 238-242.
- OSCHE, G. (1954). Über drei gegenwärtig ablaufende Entstehung von Zwillingen- und Komplementärarten bei Rhabditiden (Nematodes). (Fötilisation und Artbildung.) *Zoologische Jahrbücher. Abteilung für Systematik, Ökologie und Geographie der Tiere* 82, 618-654.
- POINAR JR, G.O. (1971). *Rhabditis adenobia* sp. n. (Nematoda: Rhabditidae) from the colleterial glands of *Oryctes monoceros* L. and other tropical dynastid beetles (Coleoptera: Scarabaeidae). *Proceedings of the Helminthological Society of Washington* 38, 99-108.
- POINAR JR, G.O. (1975). *Entomogenous nematodes: A manual and host list of insect-nematode associations*. Leiden, The Netherlands, E.J. Brill, 317 pp.
- PREMACHANDRAN, D., VON MENDE, N., HUSSEY, R.S. & MCCLURE, M.A. (1988). A method for staining nematode secretions and structures. *Journal of Nematology* 20, 70-78.
- RIDDLE, D.L. (1988). The dauer larva. In: Wood, W.B. (Ed.). *The nematode Caenorhabditis elegans*. New York, NY, USA, Cold Spring Harbor Laboratory Press, pp. 393-412.
- SCHULTE, F. (1989). The association between *Rhabditis necromena* Sudhaus & Schulte, 1989 (Nematoda: Rhabditidae) and native and introduced millipedes in South Australia. *Nematologica* 35, 82-89.
- SEYMOUR, M.K. & DONCASTER, C.C. (1972). Defaecation behaviour of *Aphelenchoides blastophthorus*. *Nematologica* 18, 463-468.
- STIERNAGLE, T. (1999). Maintenance of *C. elegans*. In: Hope, I.A. (Ed.). *C. elegans: a practical approach*. New York, NY, USA, Oxford University Press, pp. 51-68.
- SUDHAUS, W. (1974). Zur Systematik, Verbreitung, Ökologie und Biologie neuer und wenig bekannter Rhabditiden (Nematoda) 2. Teil. *Zoologische Jahrbücher. Abteilung für Systematik, Ökologie und Geographie der Tiere* 101, 417-465.
- SUDHAUS, W. (1976). Vergleichende Untersuchungen zur Phylogenie, Systematik, Ökologie, Biologie und Ethologie der Rhabditidae (Nematoda). *Zoologica* 125, 1-229.

- SUDHAUS, W. & FITCH, D. (2001). Comparative studies on the phylogeny and systematics of the Rhabditidae (Nematoda). *Journal of Nematology* 33, 1-70.
- SUDHAUS, W. & HOOPER, D.J. (1994). *Rhabditis* (*Oscheius*) *guentheri* sp. n., an unusual species with reduced posterior ovary, with observations on the *Dolichura* and *Insectivora*-groups (Nematoda: Rhabditidae). *Nematologica* 40, 508-533.
- TAHSEEN, Q. (2000). A new and a known species of *Achromadora* Cobb, 1913 (Nematoda: Cyatholaimidae) from India. *Journal of Nematode Morphology and Systematics* 3, 101-108.
- WARD, S., THOMSON, N., WHITE, J.G. & BRENNER, S. (1975). Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. *Journal of Comparative Neurology* 160, 313-37.
- YEATES, G.W. (1969a). Three new Rhabditida (Nematoda) from New Zealand dune sands. *Nematologica* 15, 115-121.
- YEATES, G.W. (1969b). A note on the function of the prerectum in *Diplenteron potohikus* (Yeates, 1969). *Nematologica* 15, 430-431.
- ZELL, H. (1983). Nematoden eines Buchenwaldbodens 2. *Rhabditis silvatica* Volz 1951 (Nematoda, Rhabditida). *Carolina* 41, 127-136.