

Heterorhabditis indicus n. sp. (Rhabditida : Nematoda) from India : separation of *Heterorhabditis* spp. by infective juveniles

George O. POINAR, Jr.,* Gopala K. KARUNAKAR** and Hastings DAVID*

* Department of Entomological Sciences, University of California, Berkeley, CA 94720 USA,
and ** Sugarcane Breeding Institute, Coimbatore 641007, Tamil Nadu, India.

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Summary — *Heterorhabditis indicus* n. sp. is described from populations recovered from the sugarcane top borer, *Scirpophaga excerptalis* (Pyralidae : Lepidoptera) in the vicinity of Coimbatore, India. The new species can be separated from previously described heterorhabditids by its shorter infective stage juveniles, differences in ratio E (distance from anterior end to excretory pore divided by distance from anterior end to base of pharynx) and ratio F (body diameter of infective stage divided by length of tail) and the reduction of number and structure of the bursal papillae, especially the last two (numbers 8 and 9). Pot experiments showed that *H. indicus* n. sp. could infect larvae of the white grubs *Holotrichia serrata* and *Leucopholis lepidophora* (Scarabaeidae : Coleoptera). By growing *H. indicus* n. sp. in small drops of *Galleria* hemolymph, it was possible to produce a continuous series of hermaphrodites, without the intervention of a sexual cycle.

Résumé — *Heterorhabditis indicus* n. sp. (Nematoda : Rhabditida) provenant de l'Inde : séparation des espèces d'*Heterorhabditis* par leurs juvéniles infestants — *Heterorhabditis indicus* n. sp. est décrit à partir de populations récoltées chez la pyrale des sommets de la canne à sucre, *Scirpophaga excerptalis* (Pyralidae : Lepidoptera), au voisinage de Coimbatore, Inde. La nouvelle espèce peut être séparée des Hétérorhabditides déjà décrits par ses juvéniles infestants plus courts, des différences dans les rapports E (distance de l'avant au pore excréteur divisée par la distance de l'avant à la base du pharynx) et F (diamètre du juvénile infestant divisé par la longueur de sa queue) et par la réduction du nombre des papilles de la bursa ainsi que par leur structure différente, plus particulièrement chez les deux dernières (numéros 8 et 9). Des expériences en pots ont montré que *H. indicus* n. sp. peut infester les larves de *Holotrichia serrata* et *Leucopholis lepidophora* (Scarabaeidae : Coleoptera). En élevant *H. indicus* n. sp. dans de petites gouttes d'hémolymphe de *Galleria*, il a été possible de produire une série continue d'hermaphrodites, sans intervention d'un cycle sexuel.

Key-words : Nematodes, *Heterorhabditis*, insect parasites.

While sampling populations of the sugarcane top borer, *Scirpophaga excerptalis* (Pyralidae : Lepidoptera) used as a trap insect in soil in the vicinity of Coimbatore, one of us (G.K.) discovered larvae infected with a representative of the genus *Heterorhabditis*. Material sent to the senior author was determined to be a new species and is described hereunder as *H. indicus* n. sp. The characters of the present species that differ from previously described forms are the length of the infective stages and male secondary sexual characters. A key is presented to separate the infective juveniles of the four known species of *Heterorhabditis*.

Materials and methods

The description presented here is based on specimens removed from *Galleria mellonella* (wax moth) larvae. Infected insects were maintained at 25 °C and dissected on days 4 and 5 to recover the first generation hermaphroditic females, and on days 9 and 10 to recover the

males and second generation amphimictic females. Infective-stage juveniles were collected as they emerged from the host cadavers approximately 14 days after initial exposure.

All stages were killed in hot (55 °C) Ringer's or water, fixed in TAF and processed to glycerin for measurements. Photographs were taken with a Nikon Optiphot microscope fitted for differential interference contrast. Life cycle studies were conducted in hanging drops of *Galleria* blood to which surface sterilized infective stage juveniles were added. Pure cultures of the symbiotic bacterium (*Xenorhabdus* sp.) were obtained using the *Galleria* blood drop method (Poinar, 1966).

Heterorhabditis indicus n. sp.

(Figs. 1-3)

MEASUREMENTS

Hermaphroditic female (n = 12) : L = 2.3-3.1
(2.7 ± 1.0) mm; greatest diameter = 107-145

(132 ± 9) µm; length of stoma = 5-8 (6 ± 1) µm; width of stoma = 6-10 (8 ± 1.3) µm; ant. end to nerve ring = 104-123 (115 ± 5) µm; ant. end to excret. pore = 163-187 (173 ± 8) µm; ant. end to base of pharynx = 163-179 (172 ± 6) µm; tail length = 72-110 (92 ± 11) µm; body diam. at anus = 38-51 (44 ± 9) µm; V = 45-50 (47 ± 3); anal swelling extended from body = 5-14 (11 ± 2.1) µm.

Amphimictic female (n = 12) : L = 1.2-1.8 (1.6 ± 0.12) mm; greatest diameter = 76-113 (95 ± 15) µm; length of stoma = 4-8 (5 ± 1.8) µm; width of stoma = 5-8 (7 ± 1.7) µm; ant. end to nerve ring = 88-96 (92 ± 4) µm; ant. end to excret. pore = 118-138 (127 ± 4) µm; ant. end to base of pharynx = 120-139 (131 ± 4) µm; tail length = 66-88 (76 ± 9) µm; body diam. at anus = 22-32 (26 ± 11) µm; V = 40-53 (48 ± 9); anal swelling extended from body = 1-3 (2 ± 0.2) µm.

Male (n = 12) : L = 573-788 (721 ± 64) µm; greatest diameter = 35-46 (42 ± 7) µm; length of stoma = 2-4 (3 ± 2) µm; width of stoma = 4-6 (5 ± 1) µm; ant. end to nerve ring = 72-85 (75 ± 4) µm; ant. end to excret. pore = 109-138 (123 ± 7) µm; ant. end to base of pharynx = 93-109 (101 ± 4) µm; reflexion of testis = 35-144 (91 ± 26) µm; tail length = 24-32 (28 ± 2) µm; body diam. at cloacal opening = 19-24 (23 ± 8) µm; spicule = 35-48 (43 ± 3) µm; greatest width of spicules = 4-6 (5 ± 1.3) µm; gubernaculum = 18-23 (21 ± 3) µm; greatest width of gubernaculum = 0.6-1.2 (0.9 ± 0.1) µm; ratio length of gubernaculum/length of spicules = 0.4-0.6 (0.5 ± 0.1).

Infective 3rd stage juvenile (ensheathed; n = 25). L = 479-573 (528 ± 26) µm; greatest diameter = 19-22 (20 ± 6) µm; ant. end to nerve ring = 72-85 (82 ± 4) µm; ant. end to excret. pore = 88-107 (98 ± 7); ant. end to base of pharynx = 109-123 (117 ± 3) µm; tail length = 93-109 (101 ± 6) µm; ant. end to hemizonid = 85-98 (91 ± 4) µm; ant. end to hemizonion = 115-131 (121 ± 12) µm; length stoma = 8-11 (10 ± 2) µm; a) = 25-27 (26 ± 4); b) = 4.3-4.8 (4.5 ± 0.34); c) = 4.5-5.6 (5.3 ± 0.5); ratio D (ant. end to excret. pore/ant. end to base of pharynx) = 0.79-0.90 (0.84 ± 0.05); ratio E (ant. end to excret. pore/tail length) = 0.83-1.03 (0.94 ± 0.07); ratio F (body diam./tail length) = 0.18-0.22 (0.20 ± 0.05).

Holotype (male) L = 668 µm; greatest diam. = 38 µm; stoma length = 3 µm; stoma width = 5 µm; ant. end to nerve ring = 80 µm; ant. end to excret. pore = 120 µm; ant. end to base of pharynx = 99 µm; reflexion of testis = 83 µm; tail length = 29 µm; anal body diam. = 23 µm; spicule length = 36 µm; greatest width of spicule = 5 µm; gubernaculum length = 19 µm; width of gubernaculum = 0.9 µm.

Allotype (hermaphrodite) : L = 2.3 mm; greatest diam. = 114; stoma length = 6 µm; stoma width =

8 µm; ant. end to nerve ring = 122 µm; ant. end to excret. pore = 162 µm; ant. end to base of pharynx = 164 µm; tail length = 88 µm; anal body diam. = 42 µm; anal swelling = 13 µm.

DESCRIPTION

Adults

Head truncate to slightly rounded; six distinct protruding lips surround mouth opening (in fixed individuals usually only the tips of the lips protrude) : head with six inner lip papillae and ten outer labial and submedial papillae. Cheilorhabdions present as a refractile ring just below the lips. The remaining meso-meta and telorhabdions are fused and enclosed by the anterior portion of the pharynx. The pharynx is cylindroid, lacks a distinct metacarpus but contains an isthmus and pronounced basal bulb containing some fine striations in the valve area. Nerve ring distinct, located in the middle of the isthmus in females but near the basal bulb in the males.

Females : Hermaphrodites and amphimictic females with paired, amphidelphic reflexed ovaries. Hermaphroditic females with sperm occurring in the proximal portion of the ovotestis; amphimictic females with sperm in the proximal portion of the oviduct. Vulva of amphimictic females narrow, surrounded with copulation plug after mating. Anal region of amphimictic females and especially hermaphrodites conspicuously swollen. The anus occurs approximately at the anterior third of the anal swelling. The rectum is heavily cuticularized and a conspicuous valve separates it from the intestine.

Males : Males with a single, reflexed testis; spicules paired and separate, with pointed tips; capitulum usually set off from the shaft, which contains a single medial rib. Gubernaculum flat, narrow, approximately half the spicule length, not reflexed at tip. Bursa open, peloderan, with a double membrane, one running external and the other internal to the bursal papillae; bursa with only seven normal papillae, the last two (Nos 8 and 9) are generally atrophied, highly modified (round or very slender) or absent. From anterior to posterior, bursal pair No. 1 are normally located anterior to the spicule heads, are straight and reach the bursal rim, pairs No. 2 and 3 form a group located near the middle of the spicules. They are straight and reach the bursal rim. The fourth, fifth and sixth pairs form another group located near the cloacal opening. The fourth pair turn outward (laterally) and do not reach the bursal rim, the fifth and sixth pair turn ventrally (inward) and usually reach the inner bursal rim. The seventh pair are often branched or swollen at their base, are variable in form (straight, bent outward or inward) and may or may not reach the bursal rim. The eighth and ninth pairs are often absent but if present are modified in being short, narrow or swollen. They may also be branched and sometimes are asymmetrical with No. 8 present and No. 9 absent on one side

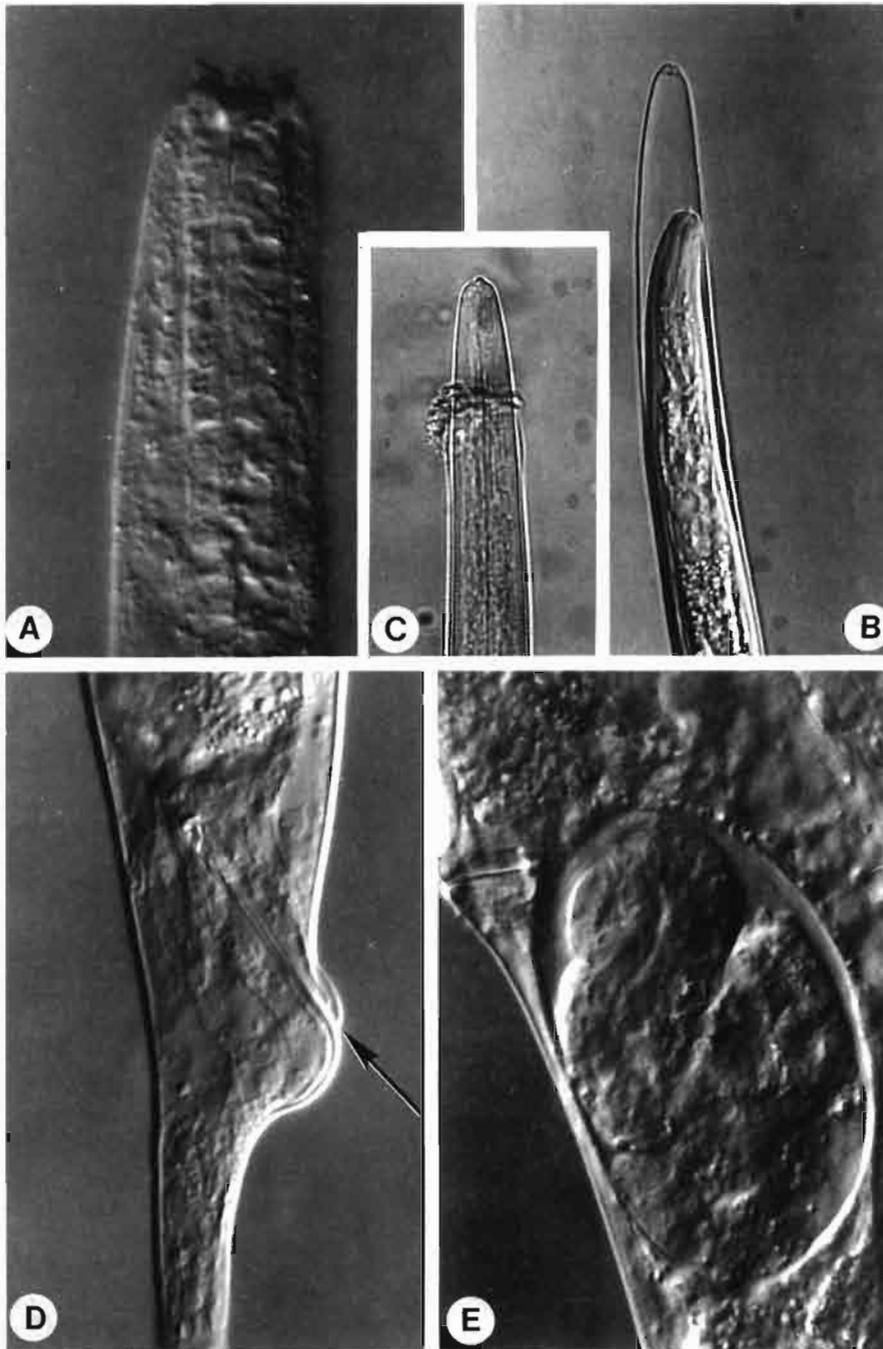


Fig. 1. *Heterorhabditis indicus* n. sp. A : Lateral view of head of amphimictic female; B : Lateral view of infective stage juvenile inside ensheathing second stage cuticle; C : Infective stage juvenile with ruptured anterior portion of ensheathing second stage cuticle; D : Lateral view of tail of hermaphroditic female showing anal opening (arrow) in anterior half of anal swelling; E : Lateral view of vulvar region of hermaphroditic female with developing egg in uterus.

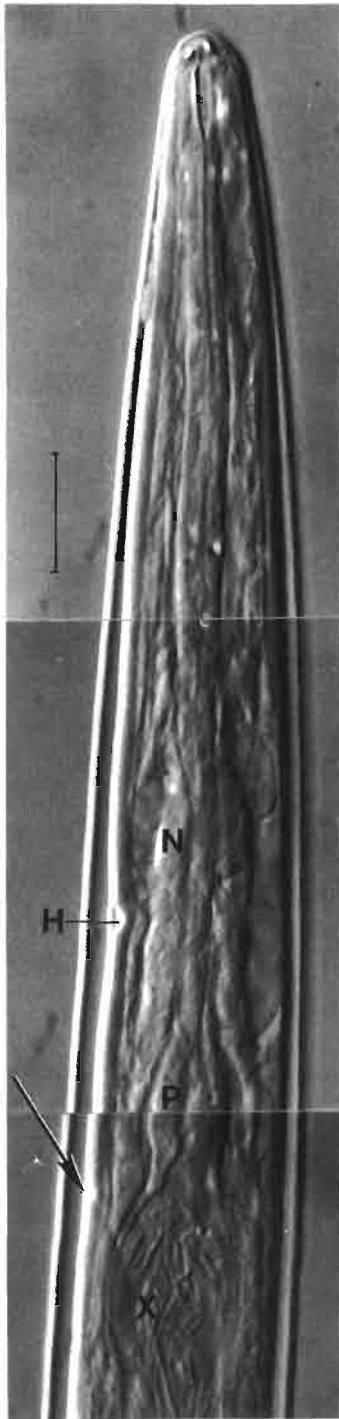


Fig. 2. *Heterorhabditis indicus* n. sp., lateral view of ensheathed infective stage juvenile. Note smaller subventral tooth (on left) and larger dorsal tooth (on right) on tip of head. N = nerve ring; H = hemizonid; P = basal bulb of pharynx; X = *Xenorhabdus* in intestinal pouch; arrow shows hemizonium (Bar = 11 μ m).

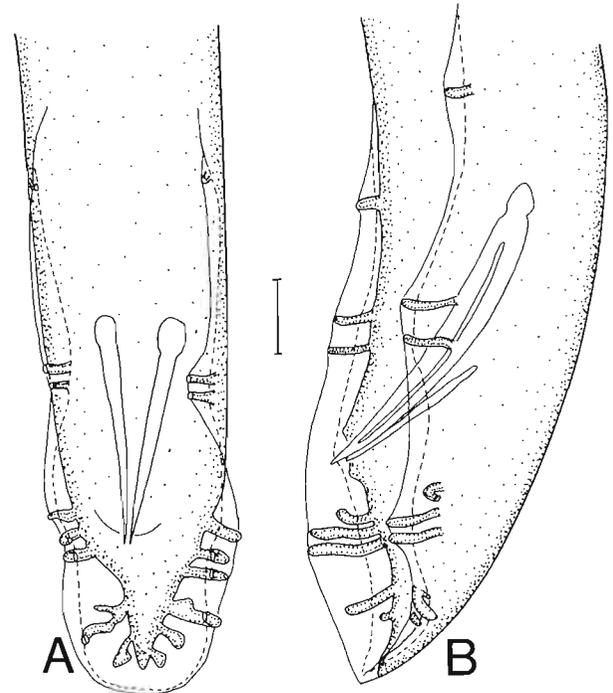


Fig. 3. *Heterorhabditis indicus* n. sp. A : Ventral view of male tail; B : Lateral view of male tail (Bar = 10 μ m).

and both present on the opposite side (always reduced in length or width) (as in Fig. 3A).

Third stage infective juveniles

The infective juveniles (third stage juveniles inside second stage cuticles) are shorter on the average than those of previously described species. The lips of the third stage juvenile contain a large dorsal tooth and two smaller but distinct subventral teeth. The stoma walls are opened and not collapsed at the base. The outer second stage cuticle is strongly ribbed longitudinally and is closely appressed to the third stage cuticle. The hemizonid is quite distinct and located just anterior to the excretory pore. The smaller less distinct hemizonion is located in the anterior intestinal region. The ventricular portion of the intestine is devoid of intestinal cells and forms an intestinal pouch which is filled with symbiotic bacteria. These bacteria also occur in the lumen of the intestine, mainly the anterior portion.

TYPE HOST AND LOCALITY

Scirpophaga excerptalis (Pyralidae : Lepidoptera) used as a trap insect in soil; Coimbatore, Tamil Nadu, India.

TYPE SPECIMENS

Holotype (male) and allotype (hermaphroditic female) deposited in the Nematology Collection at the University of California, Davis, California. Paratypes (one male,

one female) deposited in the Laboratoire des Vers, Muséum National d'Histoire Naturelle, Paris.

DIAGNOSIS

Rhabditoidea (Oerly). Heterorhabditidae Poinar, 1975 (Poinar, 1990). *Heterorhabditis indicus* n. sp. is the fourth species to be described in the genus *Heterorhabditis* Poinar and can be separated from the other three species by the key below.

The new species possesses the shortest known infective juveniles which separates it from all three described species although there is some overlap between small individuals of *H. bacteriophora* and large individuals of *H. indicus* n. sp. However, the values of ratio E (distance from head to excretory pore divided by tail length) and ratio F (body width divided by tail length) separate *H. indicus* n. sp. from all three previously described *Heterorhabditis* species. The infective stages of *H. indicus* frequently contain an intestinal pouch (ventricular portion of the intestine modified to hold cells of the symbiotic bacteria, *Xenorhabdus* sp.) which had not been noted previously in members of this genus.

KEY TO THE INFECTIVE STAGE JUVENILES

In the following key to the infective stage juveniles, the average value refers to the arithmetic mean of ten individuals. All measurements were made on ensheathed infective stage juveniles (the enclosing second stage cuticle adds approximately 40 μm to the length of the third stage juveniles).

1. — Average length less than 570 μm (479-573 μm); ratio E mean less than 1.03 (0.83-1.03); ratio F mean less than 0.22 (0.18-0.22) *H. indicus* sp. n.
— Average length greater than 570 μm (512-800 μm); ratio E mean greater than 1.03 (1.03-1.20); ratio F mean greater than 0.22 (0.22-0.36) 2.
2. — Average length less than 620 μm (512-671 μm); average distance ant. end to base of pharynx less than 135 μm (100-139 μm) *H. bacteriophora* Poinar, 1976
— Average length greater than 620 μm (570-800 μm); average distance ant. end to base of pharynx greater than 135 μm (135-160 μm) 3.
3. — Average length less than 720 μm (570-740 μm); average distance ant. end to base of pharynx 140 μm (135-147 μm) *H. zealandica* Poinar, 1990
— Average length greater than 720 μm (736-800 μm); average distance ant. end head to base of pharynx 155 μm (147-160 μm)
..... *H. megidis* Poinar, Jackson & Klein, 1987

REMARKS

The male of *H. indicus* sp. n. can be separated from *H. megidis* by its peloderan bursa (leptoderan or pseudopeloderan in *H. megidis*) and differs from all three described *Heterorhabditis* by the absence or modification of the 8th and 9th bursal rays (if present they are reduced in size, usually swollen and rarely reach the bursal rim).

BIOLOGICAL NOTES

Infective stages of *Heterorhabditis indicus* sp. n. recovered from *S. excerptalis* were used to infect larvae of the whitegrubs, *Holotrichia serrata* and *Leucopholis lepidophora* in pot experiments. Both of these insects are serious pests of sugarcane in India. The life cycle of *H. indicus* is typical for members of the genus *Heterorhabditis*. The infective stages develop into hermaphroditic females which produce young which normally mature into males and amphimictic females. Mating occurs and the young then develop into infective stages which become hermaphrodites. When the infective stages of *H. indicus* n. sp. were placed into small confined blood drops, they developed into hermaphroditic females. However, due to the restriction of available nutrients, instead of continuing the normal development into an amphimictic generation, the young developed into infective juveniles which always developed into hermaphroditic females. Thus it was possible to have a continual series of hermaphrodites (four continuous generations were obtained in the present study) without the intervention of an amphimictic cycle. However, when normal nourishment was provided, an amphimictic generation was produced.

SYMBIOTIC BACTERIA

The infective juveniles of *H. indicus* n. sp. carry bacterial cells of *Xenorhabdus luminescens* in their intestines. These cells are characteristic in shape and also occur in the hemolymph of insects invaded by the nematodes. They produce a red pigment that imparts a rufous color to the host. The bacteria luminesce and insects infected with these nematodes glow in the dark 24-48 h after death.

Discussion

This fourth described species of *Heterorhabditis* establishes the genus in India and suggests that this group has a world wide distribution. The small size, clearly modified ventricular intestinal pouch and reduced 8th and 9th pairs of bursal papillae of *H. indicus* n. sp. suggests that it may be the most highly specialized of all known *Heterorhabditis* species. Poinar *et al.* (1987) described an inner semibursa that extended only partially up the bursal papillae in *H. megidis*. This structure is probably typical for all members of the genus since it was noted for *H. bacteriophora* (Poinar & Georgis, 1990) and for *H. indicus* n. sp. in the present study. Detailed observations on the males of *H. indicus* n. sp. showed that the bursa is actually double and consists of two separate membranes, one extending along the outer edge of the bursal rays (normal bursa) and the other extending up the inner edge of the bursal rays (semibursa). The bursal papillae that bend outward hold the outer bursal membrane at a different level from the inner bursal

membrane, which normally makes contact with the bursal papillae that curve inward.

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