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HETERORHABDITIS HELIOTHIDIS, A PARASITE OF INSECT PESTS IN CUBA

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Abstract. Nematodes of the species *Heterorhabditis heliothidis* were recovered from larvae and pupae of *Pachneus litus* and *Cylas formicarius*, both dangerous pests in citrus plantations and sweet potato fields, which the nematode killed at a considerable rate. Studies on the life cycle of the parasite disclosed a typical heterogony. The bursa of the male was supported by nine pairs of papillae, its spicules and the gubernaculum were simple, direct. The incidence of the nematode species in the Caribbean region suggested that the area of its distribution covered without interruption both North- and South America. Owing to this fact the parasite might well be used in the biological control of pests in Cuba.

Members of the family Heterorhabditidae are obligatory parasites of the body cavity of insects, but knowledge of these nematodes has been made available very recently only. The species received in the family are these: *H. bacteriophora* (Poinar), *H. hambletoni* (Pereira) (syn. *Rhabditis hambletoni*), *H. heliothidis* (Khan et al.) (syn. *Chromonema heliothidis*) and *H. hoptha* (Turco) (syn. *Neoaplectana hoptha*). In addition, there is knowledge of not clearly defined species, *Heterorhabditis* spp., from Australia, New Zealand, the USA, Poland and France. Poinar (1979) gave a detailed account of the systematics and the bionomics of the four species, Wouts (1979) described the life cycle and the morphology of all developmental stages of a New Zealand population of *H. heliothidis*. Members of the family Heterorhabditidae have a similar mode of infection of the host, and kill the host at a similar rate, to members of the family Steinernematidae. Infective larvae of the parasite carry in their gut symbiotic-like bacteria which they introduce to the host (Poinar 1975). The two families can be distinguished from each other by morphological features and a slightly different life cycle. E.g., male members of the family Heterorhabditidae have a bursa, their spicules are elongate, their first generation is parthenogenetic; male members of the family Steinernematidae have no bursa, their spicules are curved, their first generation is bisexual. While in search of new biological control measures and new methods for investigating diseases of several economically important insect pests in Cuba, two nematode strains, both isolated from beetles of the family Curculionidae, were identified as members of the family Heterorhabditidae. The mouth opening of the nematodes was surrounded by six lips, the buccal cavity was short, the oesophagus cylindrical in shape, its terminal bulb well developed. The vulva of the female was indistinct, the bursa in the tail portion of the male supported by papillae. Both the spicules and the gubernaculum were simple, direct, relatively narrow. The life cycle was typically heterogonic. We studied in detail the morphology of the individual parts of the body and compared our data with all those available in the literature, and asked also Professor Poinar, Dr. Wouts, Dr. Bedding and Dr. Akhurst on their opinion of the subject. The evidence obtained suggested that the nematode species from Cuba was *Heterorhabditis heliothidis*.

Because our finding from the Caribbean region was interesting in that it confirmed a connection between the known sites of incidence of the species in North- and South America, and in Australia, we decided to add new data on the incidence of the nematode in Cuba and compare these with those on so far known and described species.

MATERIALS AND METHODS

Nematodes were obtained from larvae and pupae both of the citrus plant pest *Pachneus litus*, and the pest of sweet potato, *Cylas formicarius*. The larvae of *P. litus* feed on the roots of citrus plants, the adults on their leaves and fruit. Both larvae and adults of *C. formicarius* feed on the tubers of sweet potato and also on its leaves.

The nematode infection was clearly apparent on the infected tubers. Cut tubers were filled with deeply red, dead larvae and pupae of the host. Khan et al. (1976) reported a similar colour effect for infections caused by heterorhabditids. Infected specimens of *P. litus* were recovered from the roots of citrus plants, at 20—30 cm under the soil surface. Using Galleria traps (for method see Mráček 1980), nematodes were obtained from soil samples taken from the immediate vicinity of infected plants and trees with dead specimens of the pest. In the field, parasitism by the nematode accounted for 25—30 %. The pathogenicity of the nematode to other pest species was confirmed in preliminary, laboratory tests.

Nematodes from citrus plantations at Estación de Sanidad de Cítricos, province Havana, are designated P_2M, those from sweet potato fields in the area of Artemisa, province Havana, are designated Tetuan.

In the laboratory, larval *Galleria mellonella* were used for reproduction of the nematode. The temperature at which the nematode developed in the larval host was 26—28 °C. In spite of host mortality at room temperature, the nematode did not develop. Live individuals, immobilized by thermal shock, were used for photography and measurements.

RESULTS

We observed no morphological differences between the two strains, P_2M and Tetuan, and minimal differences in measures of the individual parts of the body. Therefore, we assigned both strains to the species *Heterorhabditis heliothidis* (Khan et al.). Measures of adults of the first and the second generation, and of infective larvae, area shown in tables 1—3.

Table 1. Comparative measurements of infective larvae of *Heterorhabditis heliothidis* strain Tetuan (n = 20)

Character	First generation		Second generation			
	Females		Females		Males	
	Mean	Range	Mean	Range	Mean	Range
Body length	2840	1320—4360	1790	1340—2240	837	663—1010
Body width	201	144—257	113	70—156	64	31—78
Stoma length	8	7—9	6	5—8	4	3—4
Stoma width	8	7—10	6	5—7	3	2—4
Length to excretory pore	164	129—199	115	108—135	95	90—101
Length of oesophagus	193	168—218	135	117—152	107	101—113
Tail length	125	86—164	98	70—125	38	31—44
Tail width	86	82—90	39	27—51	37	31—43
% vulva	46	38—54	47	41—53	—	—
Spicules					42	40—43
Gubernaculum					21	18—22

All measurements in micrometers.

Table 2. Comparative measurements of females and males of *Heterorhabditis heliothidis* strain P_2M (n = 20)

Character	First generation		Second generation			
	Females		Females		Males	
	Mean	Range	Mean	Range	Mean	Range
Body length	2555	1460—3650	1990	1250—2730	837	679—995
Body width	218	152—285	135	90—179	88	62—113
Stoma length	8	6—9	7	4—8	3	2—4
Stoma width	7	5—8	6	4—8	3	2—4
Length to excretory pore	164	133—195	124	124—124	93	93—93
Length of oesophagus	181	152—211	135	117—152	99	78—121
Tail length	131	78—183	100	82—117	37	27—47
Tail width	64	39—90	41	31—51	33	23—43
% vulva	47	39—54	48	43—53		
Spicules					46	38—54
Gubernaculum					20	16—22

All measurements in micrometers.

Table 3. Comparative measurements of infective larvae of *Heterorhabditis heliothidis* (n = 20)

	TETUAN		P_2M	
Character	Mean	Range	Mean	Range
Body length	620	550—700	610	540—680
Body width	25	20—31	25	20—31
Length of oesophagus	125	109—140	131	109—152
Tail length	111	86—137	105	98—112
Tail width	16	12—20	17	13—21

All measurements in micrometers.

DESCRIPTION OF THE FIRST GENERATION

The first generation in the host was characterized by the presence of parthenogenetic females (Fig. 1A). These were about 1.5—2 times as big as females of the second generation. Their maximum body length ranged from 4—5 mm at an average width of about 200 μm. The body was elongate in shape, the cephalic portion slightly rounded. The cuticle displayed a distinct striation, lateral fields were absent. The mouth opening was surrounded by six lips each bearing one labial papilla (Plate I, Fig. 1). Amphids opened laterally immediately under the ring of lips. The tail portion was conoidal, the opening of the anal pore was close in front of a distinct protruberance.

The stoma was reduced, short, relatively broad, the oesophagus simple, cylindrical, with a distinct, terminal bulb (Fig. 1C). The nerve ring was situated in the isthmus area close in front of the bulb. The excretory pore opened immediately below the level of the nerve ring. The intestine was sac-shaped, the ovaries paired, reflexive. The opening of the vulva was generally immediately above mid-body.

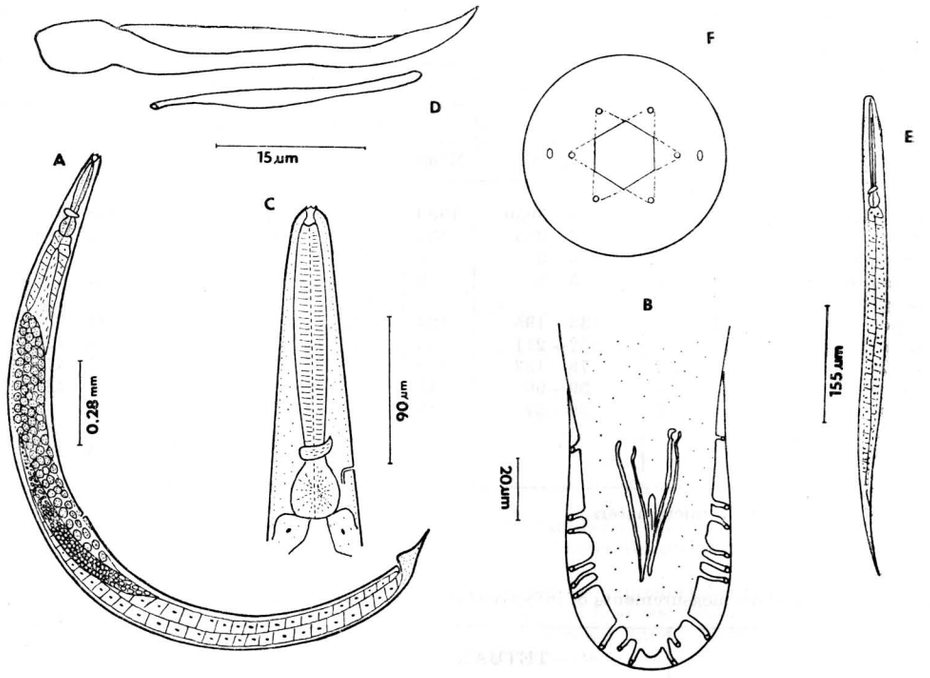

Fig. 1. *Heterorhabditis heliothidis.* A — hermaphroditic female, B — ventral view of male tail, C — oesophageal region of female, D — spicula and gubernaculum, E — infective juvenile, F — head frontal view.

DESCRIPTION OF THE SECOND GENERATION

Characteristics of females of the second generation were identical to those of the first generation except that they were considerably smaller in size measuring about 1.8 mm in length at about 120 µm in width.

The location of the internal organs and the shape of the body were identical to both sexes, also the organization of the sensory organs in the head. Testes simple, unpaired, reflexive. Differently shaped was the tail portion ranging from a convexly conoidal to a multidigital shape. Typical of this area was the presence of a peloderan bursa armed with nine pairs of papillae (Fig. 1B), three pairs of these were preanal, six pairs postanal. Preanal papillae in two groups of one and two, extended always to the margin of the bursa. Postanal papillae in two groups — three papillae each which did not always reach the bursa. Sometimes, one of the postanal papillae absent. Spicules paired, slender, straight or slightly curved. Spicule length from 38—54 µm. Gubernaculum club-shaped, about half the length of the spicule (Fig. 1D).

Infective larvae (Fig. 1E) elongate, subtile. Body length from 550—700 µm at 20—31 µm in width. Surface of cephalic portion smooth, mouth opening without distinct lips and papillae (Plate I, Fig. 2). Cuticle close under the head with transverse and longitudinal striation producing a regular network. In mid-body, areas of longitudinal striation alternated with areas of a net-like structure. Longitudinal striation on surface of tail portion (Plate II, Fig. 1). Larva L_3 almost identical in its size to L_2, but differed in the type of striation which was regular throughout the length of the body and possessed lateral fields with two longitudinal stria (Plate II, Fig. 2).

DISCUSSION

Entomophilic nematodes whose mode of infection is associated with the presence of symbiotic bacteria introduced by the parasite to the host, are highly effective biological control agents of insect pests. Their life cycles and the mode of infection are well known. Satisfactory results have been achieved with the application of mass-produced nematodes to large areas. However, there is considerable confusion in regard to the taxonomic position of several groups including the family Heterorhabditidae. The earlier descriptions of *H. hambletoni* and *H. hoptha* ought to be revised, and the result compared with detailed data on *H. bacteriophora* and *H. heliothidis*.

Our nematode species from Cuba appears to be most similar to the species *Heterorhabditis heliothidis*, e.g., in the identical site of opening of the excretory pore immediately below the level of the nerve ring. According to Poinar (1979), this site is a reliable diagnostic sign in a differentiation of *H. bacteriophora* and *H. heliothidis*. In the former, the excretory pore opens below the terminal bulb. Other similarities have been found in the size of the spicules, the gubernaculum and the length of the infective larva. However, there are other inconsistencies. Wouts (1979) assigned a population from New Zealand to the species *H. heliothidis* in spite of a different position of the opening of the excretory pore changing in the individual stages and sexes. Although the opening of the excretory pore could be discerned in several specimens only of our Cuban material, its location was always in agreement with that given by Poinar (1979) and Khan et al. (1976) for *H. heliothidis*. It will be necessary to confirm the reliability of this diagnostic sign in further, detailed studies of all described species.

Of importance mainly in generic characteristics appears to be the surface structure of the cuticle. The mouth opening bears a single circle of labial papillae, but its shape is hexagonal. While the number and the arrangement of caudal papillae was constant in our material, that of the postanal papillae was not, because frequently one was missing.

Table 4 shows several important taxonomic data on species received in the family Heterorhabditidae. There are minimal differences in the individual species. The number of pairs (12) of anal papillae reported for the species *H. hoptha* ought to be revised because the description of the species is incomplete. Apart from the location of the opening of the excretory pore, the species *H. bacteriophora* does not differ from *H. heliothidis*. Differences in the length of infective larvae are not marked.

Most complicated is the life cycle and the development, and that of the larva in particular. Wouts (1979) examining into great detail a population of *H. heliothidis* from New Zealand, determined most accurately differences in L_2 and L_3 even without scanning electron microscopy. He regarded L_2 as the infective larva (formerly L_3). Our results are in full support of his statement: The majority of nematodes in our water traps displayed a longitudinal striation and a network-like structure (L_2), those with lateral fields were extremely rare (L_3).

The ecology of the family and its zoogeographical distribution are most interesting. It is distributed both in tropical and subtropical regions of the temperate zone in America, Australia and Europe, and will certainly be found also in other continents. The nematodes are well adaptable to various temperatures. The tropical strain of *H. heliothidis* from Cuba does not reproduce in the laboratory at about 20 °C, infective larvae stored at 5—8 °C in the refrigerator, die. On the other hand, the New Zealand strain of *H. heliothidis* (temperate zone) adapted to these temperatures,

Table 4. The comparison of some characters in species of the family Heterorhabditidae

	Spicules length	Gubernaculum length
H. bacteriophora	40 (36—44)	20 (18—25)
H. hoptha	47 (43—60)	28 (26—30)
H. hambletoni	41	20
H. heliothidis (USA)	44 (37—57)	24 (21—25)
H. heliothidis (NZ)	51 (48—55)	22 (19—25)
H. heliothidis (Cuban)	44 (38—54)	21 (16—22)

p — pairs, x — second generation, NZ — New Zealand.

Table 4 (continued)

Anal papillae (pre-	ad-	post-)	% vulva	Infective larvae
9p (3	0	6)	47x (42—53)	570 (520—600)
12p (4	2	6)	47x	
9p (3	0	6)	50x	467
9p (3	0	6)	50x (48—53)	644 (619—671)
9p (3	0	6)		685 (570—740)
9p (3	0	6)	48x (41—53)	615 (540—700)

grows, reproduces and survives for a considerable length of time. Our next studies will be concerned with systematics, the ecology and possibilities for mass reproduction of these nematodes.

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HETERORHABDITIS HELIOTHIDIS, ПАРАЗИТ ВРЕДНЫХ НАСЕКОМЫХ НА КУБЕ

Е. М. Артеага Хернандез и З. Мрачек

Резюме. Нематоды вида *Heterorhabditis heliothidis* выделялись из личинок и куколок *Pachneus litus* и *Cylas formicarius*, вредных насекомых на цитрусовых плантациях и полях под сладким картофелем, где эти насекомые причиняют большие убитки. При изучении жизненного цикла паразита обнаружена типичная гетерогония. Бурса самца имеет девять пар сосочек, спикула и губернакулум несложные, прямые. Встречаемость этого вида нематод в Карибской области свидетельствует о том, что область его распределения покрывает непрерывно Северную и Южную Америку. Следовательно, этого паразита можно использовать для биологической борьбы с вредными насекомыми на Кубе.

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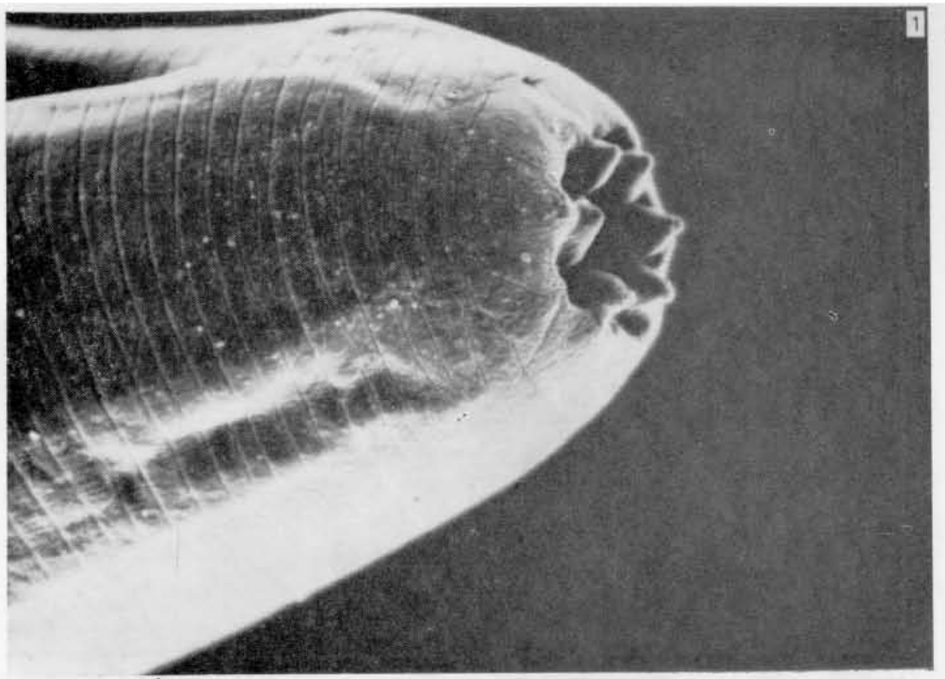


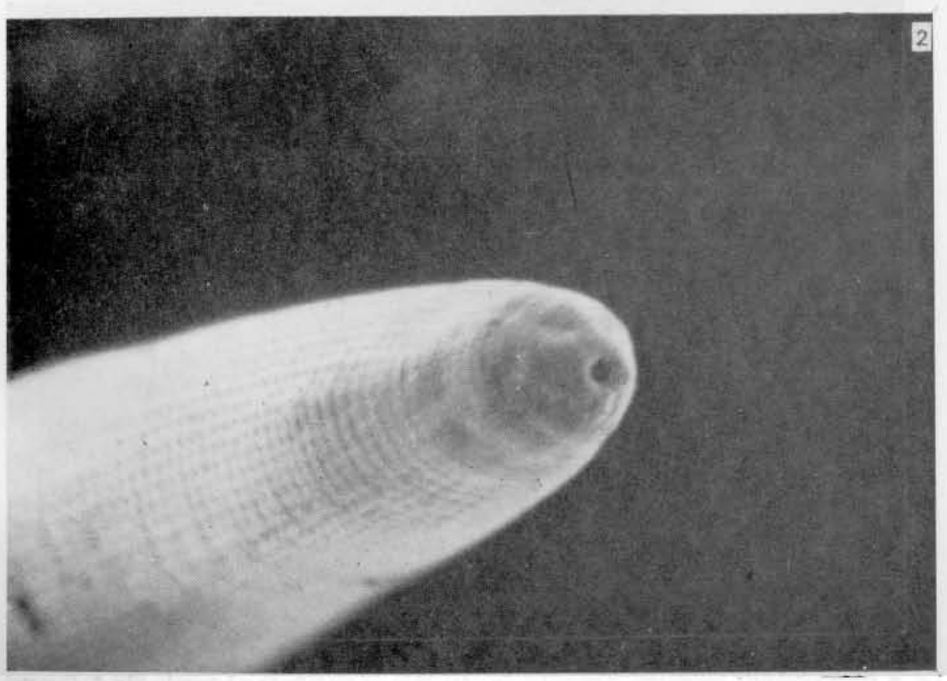
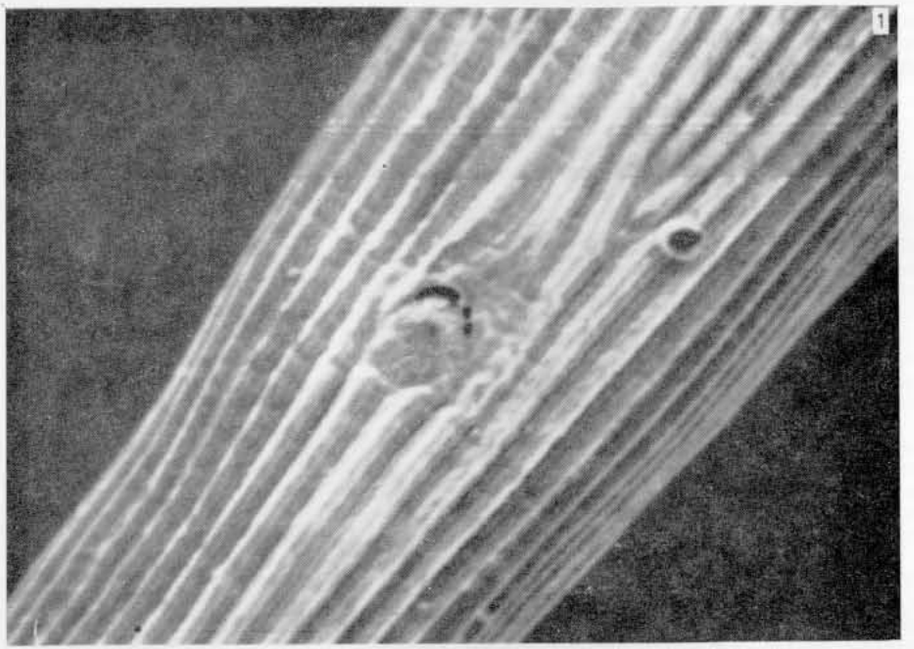


Fig. 1. ***Heterorhabditis heliothidis.*** The head of a hermaphroditic female. **Fig. 2.** ***Heterorhabditis heliothidis.*** The head of an infective juvenile.



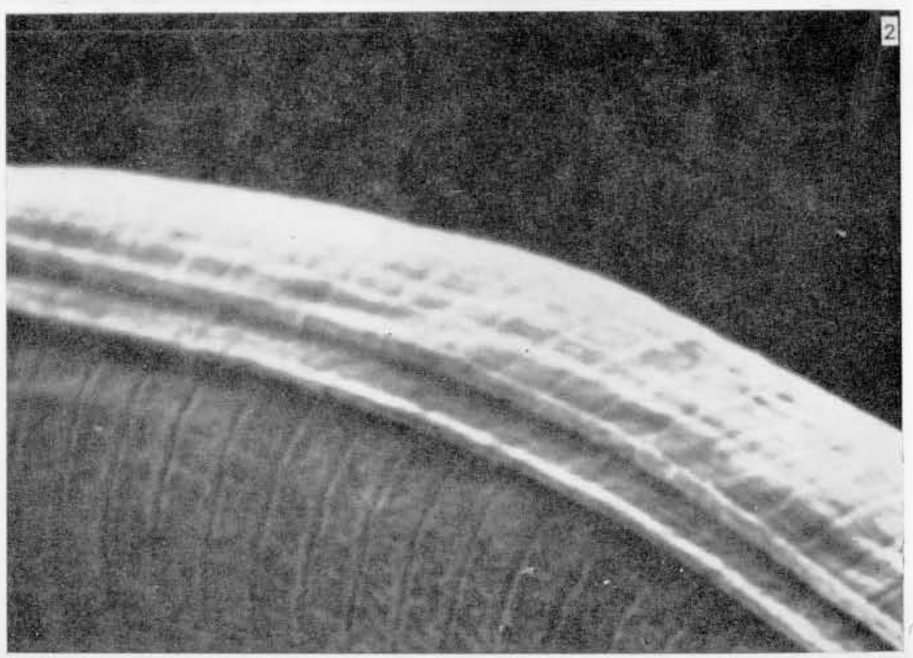


Fig. 1. *Heterorhabditis heliothidis.* The cuticular striation of an infective juvenile. **Fig. 2.** *Heterorhabditis heliothidis.* The cuticular striation of L_3 with lateral fields.