# Chromonema heliothidis n. gen., n. sp. (Steinernematidae, Nematoda), a Parasite of Heliothis zea (Noctuidae, Lepidoptera), and Other Insects[1]

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*Abstract: Chromonema heliothidis* n. gen., n. sp. is described as an entomophilic nematode of *Heliothis zea* and other lepidopterous larvae; the diagnosis of the family Steinernematidae is emended. In most morphological and host-parasite features, this nematode is similar to neoaplectanid nematodes; however, males are different in having a peloderan bursa and straight to slightly curved spicules. Although the infective-stage juveniles only give rise to hermaphrodites, the nematode is hetergonic, with both males and females being produced in the second generation. Parasitized hosts are brick-red in color and luminescent in the dark because of the association of a chromogenic, bioluminescent bacterium with the nematode. The nematode is capable of parasitizing a wide range of insects with lepidopterous larvae being most susceptible. *Key Words: entomophilic nematode, morphology, taxonomy.*

An entomophilic nematode was recovered from prepupal and pupal specimens of the corn earworm, *Heliothis zea* (Boddie), from Clayton, North Carolina in the fall of 1971. The parasitic nature of this nematode was ascertained under laboratory conditions by exposing larvae of *H. zea* and *Galleria mellonella* (L.) to juveniles of the nematode. The exposed larvae died within 48 h and turned reddish-brown to dark red in color. The infected cadavers retained their coloration and did not putrefy prior to the emergence of juvenile nematodes. Such characteristics are considered typical of insects infected with neoaplectanid nematodes (11, 13).

Although the host-parasite relationship was similar to that of various neoaplectanid infections of insects, morphological examinations of the otherwise similar adults revealed that males differed significantly by the presence of a rhabditoid bursa. Such copulatory structures have not been reported in neoaplectanids.

There are only a few published works on similar entomophilic nematodes. Littig and Swain (8) described a nematode parasite of the white-fringed beetle, *Pantomorus peregrinus* Buch., which was referred to by the accession number 41088. *Rhabditis hambletoni* Pereira was described from larvae of the curculionid, *Eutinobothrus brasilienis* (Hamb.) from Brazil (9). More recently, Laumond (7) published a short note on a nematode found in France as a parasite of a curculionid of the genus *Ceutorrhynchus*.

The neoaplectanids usually are assigned to the family Steinernematidae Chitwood and Chitwood, 1937 (2). Although the family originally contained the two genera *Neoaplectana* Steiner, 1929 and *Steinernema* Travassos, 1927, Skrjabin et al. (16) indicated that *Steinernema* was a junior synonym of the genus *Oxysomatium* Railliet and Henry, 1913. Since this change left only the genus *Neoaplectana* in the family Steinernematidae, Sobolev (17) renamed the taxon Neoaplectanidae. Turco et al. (21) provided an emended diagnosis of the family, but few nematologists have accepted Neoaplectanidae as a valid taxon. Welch (24) indicated that his prior use of this taxon was incorrect, and most nematologists continue to use the taxon Steinernematidae (1, 10, 12, 18, 22, 23). Since the genera *Oxysomatium* and *Steinernema* can be readily differentiated on the basis of the number of cephalic papillae, the presence or absence of a gubernaculum, and the position of the female vulva (6, 22, 26), we consider *Steinernema* as a valid genus. Thus, the taxonomic status of our new species will be

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considered in the context of the delimitations of the family Steinernematidae and other families of the superfamily Rhabditoidea.

The family Steinernematidae is distinguished from other families of the Rhabditoidea by the presence of indistinct lips and a reduced stoma, esophagus with a simple corpus, indistinct isthmus and reduced muscular bulb, female amphidelphic, males without caudal alae, and with genital papillae in paired preanal series. All members of the family are known as parasites of insects. Although only a single species in the genus *Steinernema* has been described, estimates of the number of species in the genus *Neoaplectana* range from 14 (21) to 19 (18); moreover, Stanuszek (18) recently revised the genus and suggested that all the known species be reduced to only three species. None of the described species, however, appear to have long genital papillae or caudal alae except possibly *N. hoptha* Turco, 1970. Males of this species were described (21) as possessing numerous setae, (possible genital papillae-?), but no mention of setae was made in the original description of the species (20).

The present paper is concerned with the description and life history of *Chromonema heliothidis* n. gen., n. sp. and its taxonomic status as an entomophilic nematode.

## MATERIALS AND METHODS

Preliminary studies on the nematode were carried out with the strain originally isolated in 1971 from dead prepupal and pupal speciments of *H. zea*, which had been recovered from pupation chambers filled with field-collected soil. This isolate, however, was lost during prolonged storage under refrigeration, and a new isolate was recovered subsequently from soil samples taken from the same field location approximately 1 year later. A few nematode-infected larvae were found when late-stage *H. zea* larvae were allowed to pupate in this field soil. The new nematode isolate used throughout this study, however, was obtained by screening the soil samples according to the Baermann funnel method (15). Subsequently, larvae of *H. zea* were exposed on artificial diet to concentrated numbers of the various, unknown nematode species that had been earlier isolated. Corn earworm larvae with typical signs and symptoms of nematode infection were held in a modified White nematode trap (25) until juvenile nematodes emerged. Additional larvae were exposed to these juveniles, and a pure isolate of the nematode was obtained subsequently by exposing larvae of *G. mellonella* to juveniles derived from a single mature female.

Semipermanent mounts of nematodes were made in 2% formalin. Permanent mounts in glycerin were prepared according to Thorne (19). Measurements were taken of 3- and 6-day-old, first-generation females, 6-day-old males and second-generation females (time interval based on number of days following death of the host larvae, usually within 48 h of exposure at 27 C).

The sex ratio was determined from nematodes obtained on the 6th day after death of the host when mature males were most prevalent. The ratio was based on at least five counts of nematodes from each of 10 infected larvae of *G. mellonella*.

Observations on the life cycle of the nematode were made with infected, 5th-stage larvae of *G. mellonella* held at 23.5 C. The infected hosts were obtained by placing 10 larvae in each of 12 Petri dishes containing two layers of filter paper soaked in 0.25% formalin and about 3,000 infective juveniles of the nematode. At 24-h intervals, 6-10 larvae were dissected and examined for the various developmental stages of the nematode. The experiment was repeated and final observations were based on an analysis of the combined results.

Limited efforts were also made to assess the potential host range of the nematode. Larvae of *Heliothis virescens* (F.), *Manduca sexta* (Johannson), and *Estigmene acraea* (Drury) were fed artificial diet previously treated with an aqueous suspension of infective nematode juveniles. Larvae of *Argyrotaenia velutinana* (Walker), *Phormia regina* (Meigen), and *Musca domestica* L. were confined without food in Petri dishes to which the nematode inoculum was added. Larvae of *Maladera castanea* (Arrow), a white-fringed beetle (*Graphognathus* sp.), and *Culex pipiens quinquefasciatus* Say were exposed by the addition of nematode inoculum to the medium

(*i.e.* soil or water) in which the larvae were reared.

## SYSTEMATICS

FAMILY STEINERNEMATIDAE, Chitwood and Chitwood, 1937. *Diagnosis emended:* Superfamily Rhabditoidea Travassos, 1920. Lips indistinct with six labial and six cephalic papillae. Stoma reduced, short and wide. Esophagus with simple corpus, indistinct isthmus, musculature in the basal bulb reduced. Females didelphic, amphidelphic, ovaries reflexed. Males monorchic, testis reflexed; with or without caudal alae; spicules separate, curved or straight; genital papillae in paired linear series. Monogenetic parasites of insects.

Genera: *Steinernema* Travassos, 1927, *Neoaplectana* Steiner, 1929 and *Chromonema* n. gen., n. sp.

Type genus: *Steinernema* Travassos, 1927.

### *Chromonema* n. gen.

*Diagnosis:* Steinernematidae, Chitwood and Chitwood 1937. Lips indistinct, with six labial and six cephalic papillae. Stoma reduced, short and wide. Esophagus simple with indistinct isthmus; musculature in basal bulb reduced. Females didelphic, amphidelphic. Males with single reflexed testis, peloderan caudal alae with long genital papillae, and straight to slightly curved spicules.

*Type species: Chromonema heliothidis* n. sp.

*Chromonema* n. gen. differs from *Neoaplectana* and *Steinernema* by the presence of rhabditoid caudal alae with long genital papillae and straight to slightly curved spicules.

The name *Chromonema* is derived from the Greek; chroma = color and nema = nematode. The species name *heliothidis* is named after the genus of the host insect, *Heliothis.*

### *Chromonema heliothidis* n. gen., n. sp. (Figs. 1-3)

*Measurements:* (Paratypes 20 males in glycerin mounts): L = 0.87 mm (0.70-0.98); a = 18.7 (14.0-22.4); b = 7.8 (6.2-8.8); c = 27.9 (21.1-31.8); spicules = 44.4 $\mu$m (37.5-58.4); gubernaculum = 23.6 $\mu$m (20.9-25.0).

*Holotype* (male): L = 0.93 mm; a = 19.4; b = 8.2; c = 30.1; spicules = 42.9 $\mu$m; gubernaculum = 25.0 $\mu$m.

*Allotype* (female): L = 2.0 mm; a = 18.2; b = 12.4; c = 26.7; V = $^{33.1}$ 47.7 $^{25.2}$.

*Paratypes* [25 females in glycerin mounts (first generation, 3 days after death of host)]: L = 2.1 mm (1.9-2.4); a = 23.9 (16.2-27.9); b = 12.1 (10.1-14.3); c = 27.8 (20.9-34.9); V = $^{28.8}$ 46.7-52.9 $^{26.0}$.

Comparative measurements of other females and males are presented in Table 1.

*Males* (Fig. 1-B; 2-B-D; 3): Body slender, much smaller than females. Assume shape of letter J when relaxed by heat. Present for about 48 h in body cavity of host on 6th and 7th days after host's death. Head region with 6 labial and 6 cephalic papillae. Stoma reduced, short and wide; glottoid apparatus and esophageal collar lacking. Cheilo-, proto-, and telorhabdions vestigial. Esophagus with simple corpus, indistinct isthmus, and basal bulb with reduced musculature. Basal bulb valve inconspicuous. Nerve ring encircles isthmus close to basal bulb. Opening of excretory pore slightly posteriad of nerve ring. Testis single, reflexed. Spicules paired, straight to slightly curved, separate but contiguous distally. Gubernaculum half as long as spicules. Bursa peloderan, open, hyaline, with 3 pairs of preanal, and 6 pairs of postanal papillae. Bursa best seen in live specimens or in semipermanent formalin mounts under phase microscopy (Fig. 3). Sex ratio of males to females about 1:5, *i.e.*, 18.3% of the nematode population on the 6th day after death of host composed of males.

*Females* (Fig. 1-A, C, D, F; 2-A): Body robust, glistening white; assume shape of letter C when relaxed by heat. Present only in body cavity of host. Immobile, or very sluggish in movement when touched. Six conoid, labial papillae more prominent than in males. Stoma similar in shape, but slightly more cuticularized than in males. Cheilorhabdions represented as lightly sclerotized areas lining inside of lip region. Pro-, meso-, meta-, and telorhabdions vestigial. A minute, tooth-like structure present in metastomal area. Esophagus slightly more muscular than that of male. Ovaries paired, opposed, and reflexed; oviducts and uteri voluminous, filled with eggs and embryos in various stages of development. First-generation females hermaphroditic;

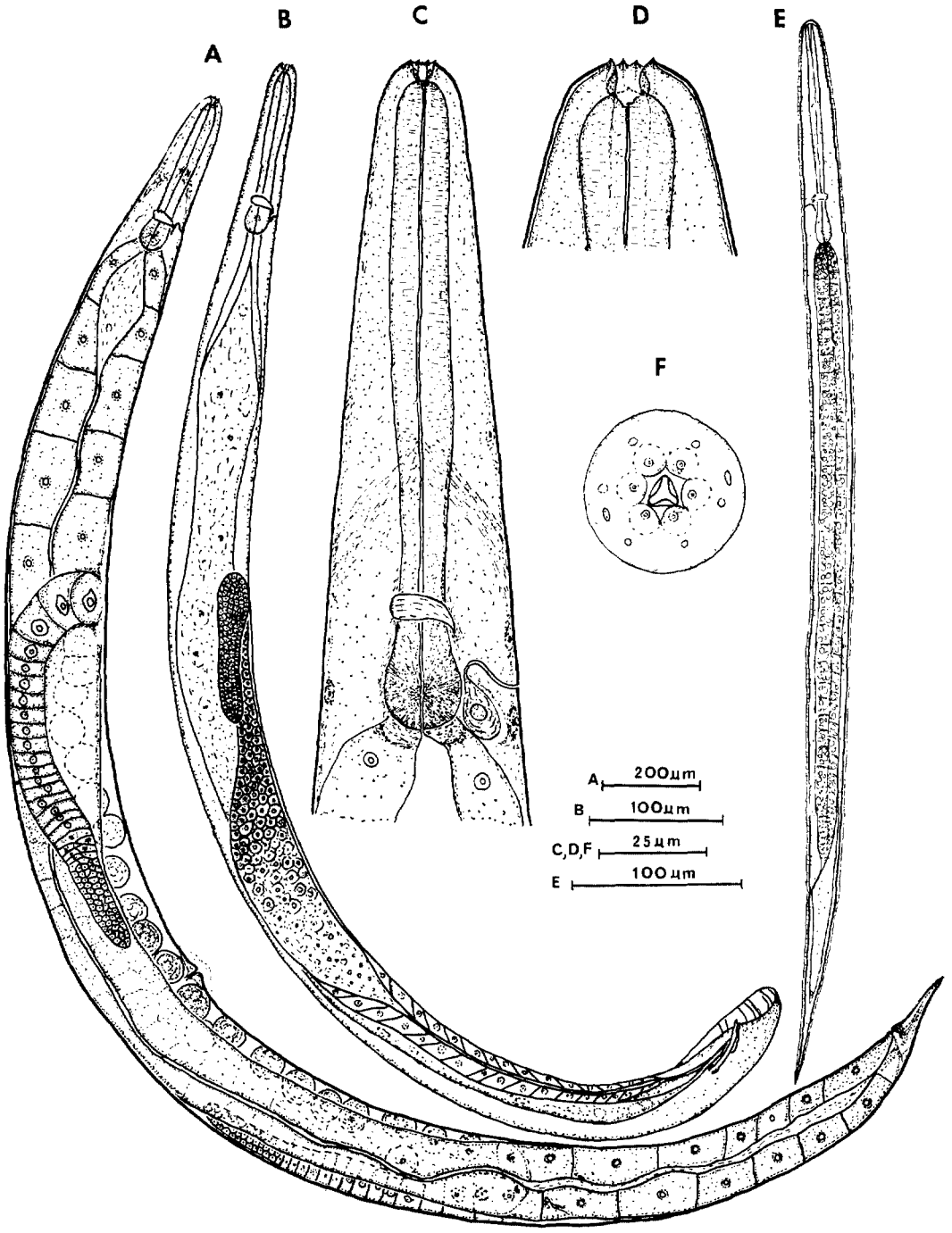


FIG. 1-(A-F). *Chromonema heliothidis* n. gen. n. sp. *A)* First-generation, hermaphroditic female. *B)* Male. *C)* Esophageal region of second-generation female. *D)* Cephalic region of first-generation female showing labial papillae and stoma. *E)* Infective juvenile. *F) En face* view of female.

the haploid chromosome number is 7 (A. C. Triantaphyllou, personal communication). Vagina muscular in younger females; vulva a transverse slit, median in location. Intestine dense, oligocytous, with rectangular cells. Three rectal glands, one dorsal and two subventral, located at junction of intestine and rectum. Anal lobe quite prom-

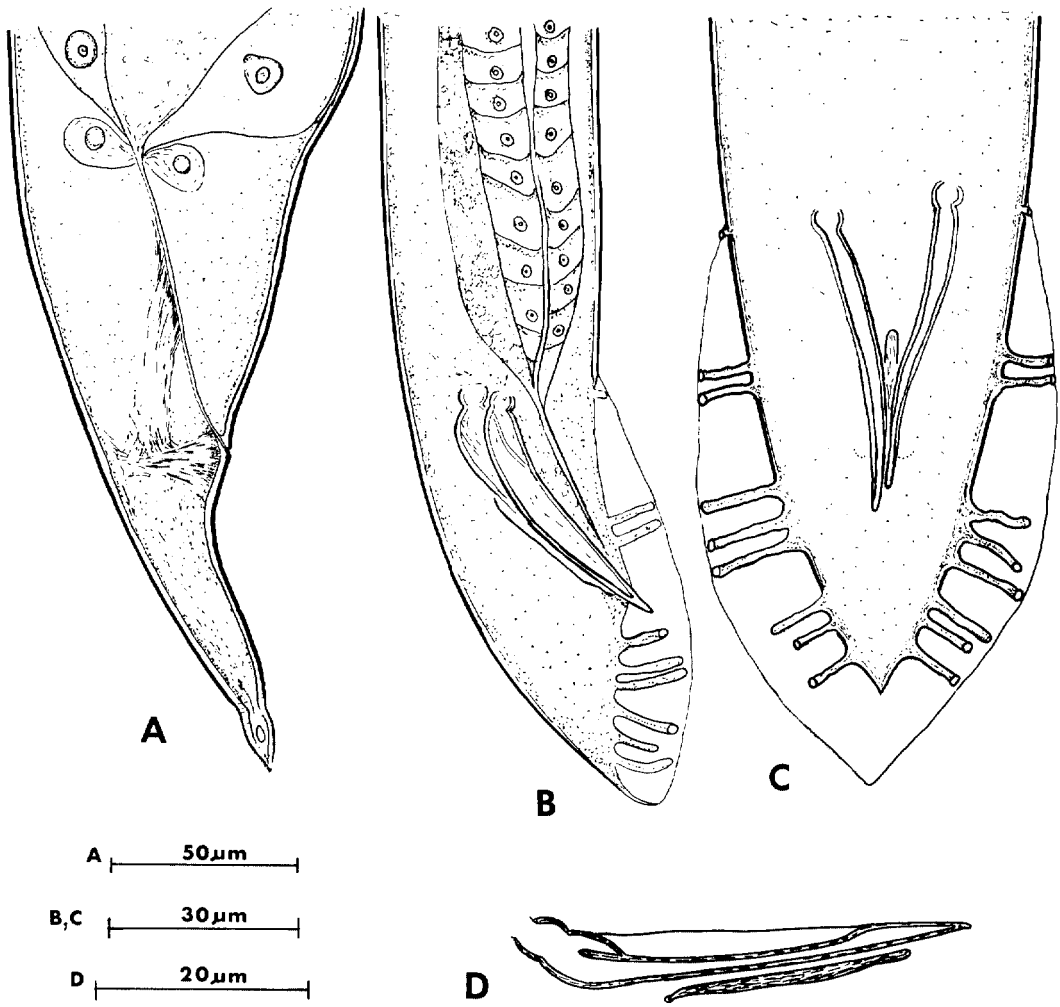


FIG. 2-(A-D). *A)* Lateral view of female tail. *B, C)* Lateral and ventral view, respectively, of male tail. *D)* Paired spicules and gubernaculum.

inent in some mature specimens (Fig. 2-A). Females differ considerably in size, particularly those of first generation (Table 1).

*Infective juveniles* (Fig. 1-E): (Measurements, n = 20): L = 644 μm (618.5-670.5); width = 25.2 μm (23.0-28.8); esophagus length = 133 μm (130-139); tail length = 108 μm (103.5-111.5); a = 26.1 (22.4-27.6); b = 4.9 (4.6-5.4); c = 6.0 (5.8-6.3). Body slender, gradually tapering posteriorly. Stoma, as opposed to that of adults, elongate and somewhat cylindrical. Mouth opening closed, digestive tract apparently nonfunctional. Esophagus long and slender. Basal bulb weak and less prominent than in adults. Location of excretory pore and nerve ring as in adults. Intestine dense, filled with lipid globules. The infective larva is a third-stage juvenile ensheathed in the cuticle of the second-stage juvenile. Sheath closely fitting, with fine longitudinal and transverse striations.

*Holotype:* Male derived from nematode culture presently maintained on wax moth larvae *G. mellonella,* in the Department of Entomology, North Carolina State University. Slide No. T-244t deposited at USDA Nematode Collection, Beltsville, Maryland.

*Allotype:* Female, same data as holotype. Slide No. T-245t deposited at USDA Nematode Collection, Beltsville, Maryland.

*Paratypes:* Same data as holotype. Males, slide nos. T-1800p-T-1801p; females, slide nos. T-1798p-T-1799p deposited at USDA

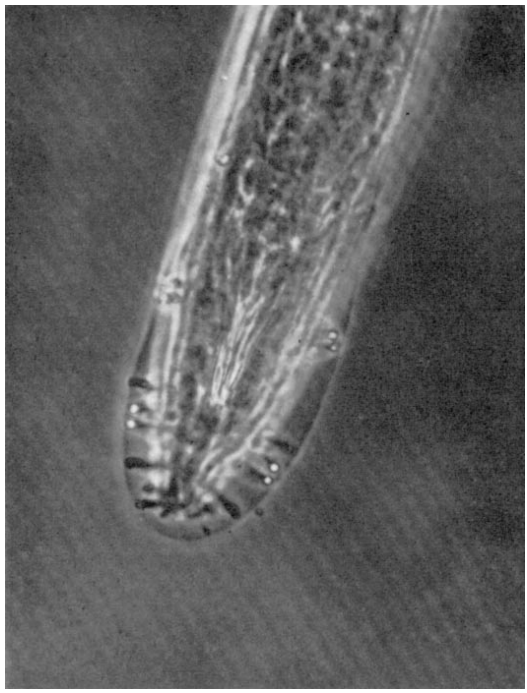

FIG. 3. Peloderan caudal alae of male with genital papillae in ventral view; phase microscopy, X 1,000.

Nematode Collection, Beltsville, Maryland. Other paratypes are stored in the collections of the authors.

*Type Host: Heliothis zea* (Boddie).

*Type Locality:* Soil, Clayton, N. C. U.S.A.

## LIFE CYCLE AND HOST-PARASITE RELATIONSHIP

The infective (ensheathed) juvenile enters the host, presumably through oral openings, and the exsheathed larva can be detected within 24 h in the host hemocoel. Host death, however, does not occur until 48-72 h after exposure and is the result of bacterial septicemia. The infective juvenile is associated with a chromogenic, bioluminescent bacterium in a manner apparently similar to that of *Achromobacter nematophilus* Poinar and Thomas and the DD-136 strain of *Neoaplectana carpocapsae* Weiser (11). As a result of the chromogenicity of the bacterium, the host cadaver gradually changes from natural to pink-red and finally to a brick-red color. The development of the brick-red color and the ability of cadavers to exhibit bioluminescence in the dark is characteristic of parasitization by this nematode-bacterial complex. The host cadaver remains intact and shows no sign of putrefaction even after the infective juveniles emerge.

The life cycle of *C. heliothidis* (Table 2) is somewhat similar to that described for various neoaplectanid nematodes (5, 11). Third- and fourth-stage, exsheathed juveniles are the only stages present in the hemocoel of cadavers at 24 and 48 h, respectively, after host death. By 3 days after host death, these juveniles give rise to oviparous females which are the first-

TABLE 1. Comparative measurements of females and males of *Chromonema heliothidis* n. gen., n. sp. in 2% formalin, 6 days after death of host, *Galleria mellonella* (L.).[a]

| Character | First Generation Females[b] (n=20) | | Second Generation Females (n=20) | | Second Generation Males (n=20) | |
|---|---|---|---|---|---|---|
| | Mean | Range | Mean | Range | Mean | Range |
| Body length (mm) | 4.2 | 3.0-5.1 | 2.3 | 2.0-3.3 | 1.1 | 1.0-1.2 |
| Body Width | 280.6 | 200.0-344.0 | 207.7 | 184.4-240.0 | 50.2 | 31.5-60.0 |
| Esophagus length | 240.8 | 163.0-286.0 | 164.2 | 147.6-177.3 | 119.5 | 112.5-130.5 |
| Tail length | 85.9 | 76.0-100.0 | 81.4 | 71.2-93.0 | 32.8 | 28.8-36.3 |
| V | 48.8 | 45.2-52.0 | 50.3 | 48.4-52.5 | | |
| a | 15.1 | 10.6-18.0 | 12.9 | 11.4-15.0 | 22.3 | 18.7-34.9 |
| b | 17.9 | 10.6-24.6 | 17.0 | 14.0-20.6 | 9.2 | 8.3-10.5 |
| c | 48.8 | 30.0-63.0 | 33.9 | 25.8-45.8 | 33.5 | 28.0-38.4 |
| Spicules | | | | | 47.3 | 41.8-52.0 |
| Gubernaculum | | | | | 24.3 | 22.0-26.8 |

[a]All measurements in micrometers unless otherwise specified.
[b]Giant ♀♀, present 7 days or later after death of host, ranged up to 7.04 mm in length.

TABLE 2. Life cycle of *Chromonema heliothidis* n. gen., n. sp. in *Galleria mellonella* at 24 C.

| Days after death of host[a] | Stages of nematode |
|---|---|
| 1-2 | Third- and fourth-stage exsheathed juveniles. |
| 3-4 | Preadult and mature, hermaphroditic females (first gen.) with eggs. Second-stage juveniles. |
| 5-6 | Giant females; preadult males and females (second gen.) |
| 7 | Giant females with juveniles; mature females and males; early-stage juveniles. |
| 8-9 | Large numbers of females (third gen.) with eggs and juveniles; dead males. |
| 10-11 | Emergence of infective ensheathed juveniles from host. |

[a]Usually 48-72 h after exposure.

generation females. The presence of sperm in the gonoducts of these females indicates that they are hermaphrodites. Eggs laid by these females account for the presence of second-stage juveniles 4 days after death of the host. The first juvenile molt of *C. heliothidis* occurs within the egg, and it is the second-stage juvenile that hatches. Although the first-generation females are hermaphroditic, the second generation consists of males and females. Males can be found in the host hemocoel 5 days after host death, and are usually present for about 48 h. The occasional males found on the 8th day have always been dead. Almost all first-generation, hermaphroditic females that are oviparous in the early stages of their life become ovoviviparous in the later stages. Such females assume a large size and are termed giant females. Juvenile nematodes, hatching within the body of these females, feed on the body contents of the mother and transform it into a sac-like structure. These juveniles finally escape through anal or oral openings of the then dead mother. Although no males are produced from the heterosexual, second generation, many juveniles derived from this generation develop into third-generation females by day 9. It is assumed that the formation of ensheathed juveniles at this time is associated with the depletion of food and accumulation of waste products within the host. The ensheathed, infective juveniles, which are apparently derived from third- and some from second-generation females, begin to emerge from the host cadaver as early as 10 days after death of the host.

Limited studies on host range (Table 3) indicate that *C. heliothidis* is capable of parasitizing a wide range of insects, with lepidopterous larvae being most susceptible. Signs and symptoms of infection in various hosts are generally similar to those previously described in this study for *H. zea* and *G. mellonella*.

## DISCUSSION

*Chromonema heliothidis* kills its host in a manner similar to neoaplectanid nematodes, and resembles members of the genus *Neoaplectana* in such morphological features as indistinct lips, a reduced stoma, esophagus with simple corpus, indistinct isthmus, and reduced musculature in the basal bulb, as well as paired, opposed gonads of females. However, the presence of caudal alae with long genital papillae in males of this species merits a separate taxon at the generic level and suggests rhabditoid affinities.

Males with caudal alae and long genital papillae are most common in the families

TABLE 3. Host range of *Chromonema heliothidis* n. gen., n. sp.

| Host Species[a] | Susceptibility[b] |
|---|---|
| Lepidoptera | |
| *Galleria mellonella* | (++) |
| *Heliothis zea* | (++) |
| *Heliothis virescens* | (++) |
| *Argyrotaenia velutinana* | (++) |
| *Manduca sexta* | (++) |
| *Estigmene acraea* | (++) |
| Coleoptera | |
| *Maladera castanea* | (+) |
| *Graphognathus* sp. | (+) |
| Diptera | |
| *Culex pipiens quinquefasciatus* | (+) |
| *Musca domestica* | (−) |
| *Phormia regina* | (±) |

[a]Larval stage.
[b](++) = Susceptible; (+) = Moderately susceptible; (±) = Doubtful; (−) = Resistant.

Rhabditidae and Angiostomatidae. *Chromonema* differs from members of these families by the absence of distinct lips; a cylindrical, sclerotized stoma; a pharyngeal collar; and a muscular valvated bulb. As the most important criteria for the recognition of rhabditoid families lie in the nature of the stoma and the esophagus (4), and as caudal alae may or may not be present among closely related genera of nematodes (3), we consider *Chromonema* to be related to the genus *Neoaplectana,* family Steinernematidae.

Little published work is available on nematodes similar to *C. heliothidis.* Pereira (9) described a nematode semiparasitic on larvae of *E. brasiliensis* from Brazil and tentatively named it *Rhabditis hambletoni.* Although the stomatal and esophageal structures were not adequately described, the apparent absence of a valvated, basal bulb and a cylindrical, sclerotized stoma suggest that this species is not a typical member of the family Rhabditidae. Except for differences in the size of various stages of the nematode, including the spicules, *R. hambletoni* appears to be generally similar in morphology and life-cycle to *C. heliothidis.* However, bacteria were not reported to be associated with *R. hambletoni* and there was no mention of any characteristic color produced in parasitized hosts. More recently, Laumond (7) mentioned the association of bacteria with an entomophilic nematode which also has a life cycle similar to *R. hambletoni.* However, as Laumond's nematode has not yet been described, it is impossible to speculate on the possible conspecificities of the nematodes involved. Of particular importance to the possible relationship of *R. hambletoni* and *C. heliothidis* is the semiparasitic nature of *R. hambletoni.* This species, in contrast to the definite parasitic nature of *C. heliothidis,* was only able to invade and reproduce in hosts with reduced vitality.

In an unpublished report, Littig and Swain (8) described a nematode parasite (referred to as accession no. 41088) of the white fringed beetle, *P. peregrinus.* This nematode is similar to *C. heliothidis* in its morphological features and host-parasite relationships. Males were described with caudal alae; bacteria were found to be associated with the nematode; and the parasitized hosts became pink to dark-brown in color. However, because of the nature of this report (unpublished) and the lack of preserved specimens available for study, no definite conclusion can be offered as to the possible conspecificity of this nematode with *C. heliothidis.*

Our attention was also drawn to *Neoaplectana hoptha* since this species, in contrast to the distinctly curved spicules of most species of *Neoaplectana,* has straight to slightly curved spicules (20). We examined paratype slides T-1275p and T-1276p of *N. hoptha* male and female specimens from the U.S.D.A. nematode collection, Beltsville, Maryland, and noticed that *N. hoptha* males not only have spicules similar in shape to those of *C. heliothidis* but that they also have long genital papillae as opposed to the nipple-shaped papillae of other neoaplectanid species. These paratype specimens of *N. hoptha* were not in very good condition, and the number and position of genital papillae, as well as the presence of caudal alae, could not be definitely ascertained. However, it is assumed that the presence of long genital papillae in *N. hoptha* suggests that peloderan caudal alae are present in this species. In this respect, it is not always possible to discern the caudal alae in permanent glycerine preparations of *C. heliothidis,* although they can be readily seen in wet-mount preparations (Fig. 3). As there are no live specimens of *N. hoptha* available and nothing is known about the biology and host-parasite relationship of this species, further analysis as to the possible conspecificity of *N. hoptha* and *C. heliothidis* is also impossible.

From the discussion presented in this study, it is obvious that *C. heliothidis* shares similar morphological features and/or life-cycle aspects with *R. hambletoni,* nematode 41088, and *N. hoptha.* However, there are sufficient reasons to question the possible conspecificities of the nematodes involved to justify our description of *C. heliothidis* as a new and distinct species. At the same time, it should be pointed out that the other nematodes are sufficiently similar morphologically to merit their possible inclusion in the genus *Chromonema,* and that further study of new isolates of these species may reveal that one or more are conspecific with *C. heliothidis.*

It might also be interesting to examine the relationship of *Rhabditis dentata* (Schneider) to these nematodes since Polozhentsev and Artyukhovskii (14) suggested that *R. dentata* is probably a neoaplectanid-like nematode.

Although we have only isolated *C. heliothidis* from a single field in Johnston Co., N. C., this entomophilic species may be widespread in the soils of the southeastern U. S. According to Dr. G. R. Carner (personal commun.), a similar, if not identical, nematode was found as a parasite of the larvae of the peach tree borer, *Sanninoidea exitiosa* (Say), at Edgefield, S. C., which was also parasitic for *Trichoplusia ni* (Hbn.), *H. zea* and *G. mellonella*. Parasitized larvae exhibited similar signs and symptoms of infection, particularly with respect to the bioluminescence and development of a brick-red color in infected cadavers. Although this isolate has since been lost, males possessed peloderan caudal alae similar to those described herein for *C. heliothidis*.

We have also examined an isolate (apparently identical to *C. heliothidis*) being maintained by Dr. John J. Hamm (USDA, ARS, Southern Grain Insects Research Lab., Tifton, Ga.) on larvae of *H. zea*. This strain was originally found parasitizing a larva of the bill bug, *Sphenophorus coesifrons* (Gyllenhal), by Dr. W. L. Morrill (Department of Entomology, University of Georgia, Experiment, Ga.) in Seminole Co., Georgia, in 1974. Although we have found a few differences in the biochemical and cultural characteristics of the bacterium associated with this nematode that are indicative of strain variation, males and females of this nematode isolate are quite similar morphologically to *C. heliothidis*.

## LITERATURE CITED


1. BENHAM, G. S., JR. 1974. A synopsis of the obligate and facultative insect parasitic nematodes. J. Invertebr. Pathol. 24:263-270.
2. CHITWOOD, B. G., and M. B. CHITWOOD. 1937. An introduction to nematology. Monumental Printing Co., Baltimore. 372 p.
3. CHITWOOD, B. G., and M. B. CHITWOOD. 1950. An introduction to nematology (Revised Edition). Monumental Printing Co., Baltimore. 213 p.
4. DOUGHERTY, E. C. 1951. Evolution of zooparasitic groups in the phylum Nematoda, with special reference to host-distribution. J. Parasitol. 37:353-378.
5. DUTKY, S. R. 1959. Insect microbiology. Adv. Appl. Microbiol. 1:175-200.
6. FILIPJEV, I. N., and J. H. SCHUURMANS STEKHOVEN, JR. 1941. A manual of agricultural helminthology. E. J. Brill, Leiden. 878 p.
7. LAUMOND, C. 1974. Alternation of bisexual and parthenogenetic generations in the cycle of a new species of bacterial vector entomoparasitic Rhabditidae. (Abstr.) 12th Int. Symposium of Nematology, Granada.
8. LITTIG, K. S., and R. B. SWAIN. 1943. Studies on nematode 41088, a nematode parasite of the white-fringed beetles. Unpub. Rep. U. S. Dep. Agric. Gulfport, Mississippi. 17 p.
9. PEREIRA, C. 1937. Rhabditis hambletoni n. sp., nema apparentemente semiparasito da "broca do algodoeiro" (Gasterocercodes brasiliensis). Arch. Inst. Biol. (Sao Paulo). 8:215-230.
10. POINAR, G. O., JR. 1967. Description and taxonomic position of the DD-136 nematode (Steinernematidae, Rhabditoidea) and its relationship to Neoaplectana carpocapsae Weiser. Proc. Helminthol. Soc. Wash. 34:199-209.
11. POINAR, G. O., JR. 1971. Use of nematodes for microbial control of insects. Pages 181-203 *in* H. D. Burges and N. W. Hussey, eds. Microbial control of insects and mites. Academic Press. N. Y.
12. POINAR, G. O., JR. 1972. Nematodes as facultative parasites of insects. Annu. Rev. Entomol. 17:103-122.
13. POINAR, G. O., JR., and G. M. THOMAS. 1967. The nature of Achromobacter nematophilus as an insect pathogen. J. Invertebr. Pathol. 9:510-514.
14. POLOZHENTSEV, P. A., and A. K. ARTYUKHOVSKII. 1963. [On the investigation of nematodes of Rhabditis and Neoaplectana in the control of pests insects] Materaly Nauchn. Konf. Vsesoyuznogo Obschchestva Gel'montologov, Chast II:46-47 (In Russian).
15. POWELL, W. M., and J. C. NUSBAUM. 1963. Investigations on the estimation of plant parasitic nematode populations for advisory purposes. N. C. Agric. Exp. Stn. Tech. Bull. 156.21 p.
16. SKRJABIN, K. I., N. P. SHIKHOBALOVA, N. P., and A. A. MOZGOVOI. 1951. A key to parasitic nematodes. 2. Oxyruates and Ascaridates. Moskva, Leningrad. 631 pp. (In Russian).
17. SOBOLEV, A. A. 1953. To define more accurately the information on Rhabditida (superfamilies Rhabditoidea and Aphelenchoidea) parasitic in insects. (In Russian) Raboty po Gel'mintel. 75-Let. Skrjabin, pp. 676-684. (See Helminthol. Abstr. 22, no. 996.)
18. STANUSZEK, S. 1972. Revision of the genus Neoaplectana Steiner, 1929 (Rhabditoidea: Steinernematidae). Pages 69-70 *in* International symposium of nematology (11th) European Society of Nematologists. Reading, U. K.
19. THORNE, G. 1961. Principles of nematology.

McGraw-Hill Book Co., Inc. N. Y. 553 pp.

20. TURCO, C. P. 1970. Neoaplectana hoptha sp. n. (Neoaplectanida Nematoda) a parasite of the Japanese beetle, Popilla japonica. Proc. Helminthol. Soc. Wash. 37:119-121.
21. TURCO, C. P., W. H. THAMES, JR., and S. H. HOPKINS. 1971. On the taxonomic status and comparative morphology of the species of the genus Neoaplectana Steiner. (Neoaplectanidae: Nematoda). Proc. Helminthol. Soc. Wash. 28: 68-79.
22. VEREMCHUK, G. V. 1962. [Entomohelminthological family Steinernematidae Chitwood and Chitwood, 1937] Tezisy Dokladov, Nauch. Konf. Vsesoyuznogo Obschchestva Gel'mintologov. Akad. Nauk, S.S.S.R., Chast I:24-25 (In Russian).
23. VEREMCHUK, G. V. 1969. Anatomical and morphological characters of Neoaplectana [Rhabditida, Steinernematidae] Mater Nauch. Konf. Vses. Obshch. Gel'mint. Part II. pp. 152-158 (In Russian).
24. WELCH, H. E. 1965. Entomophilic nematodes. Annu. Rev. Entomol. 10:275-302.
25. WHITE, G. F. 1929. A method of obtaining infective nematode larvae from cultures. Science 66:302-303.
26. YAMAGUTI, S. 1961. Systema helminthum. Vol. III. Part I. The nematodes of vertebrates. Interscience Publishers, Inc. N. Y., London. 679 p.