Biology, Life Cycle and Redescription of Neoaplectana bibionis Bovien, 1937 (Nematoda: Steinernematidae)

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Abstract: A greater knowledge of the morphology and the development of the infective third larval stage of *Neoaplectana bibionis* Bovien, 1937, a parasite of arthropods, makes it possible to recognise the second larval stage and present a description of all the developmental stages in the life cycle of the species. Four larval stages can be recognised. L1 hatches from the egg. In a suitable host, when the population density is low, it develops directly into L4. When the population density is high, it develops into L2. The L2 is a non-feeding stage that precedes the resistant infective L3 larvae. L3 is free-living and characterised by a distinct lateral field with nine longitudinal lines. In the L4 the sex can be recognised. The first adults developing in a fresh host are usually larger than those developing later. Generally no more than two generations are completed in one insect. Young females in a fresh host lay eggs. In older females, and in females developing in a decaying host, *endotokia matricida* is common. A redescription of the species is presented on the basis of a population from New Zealand reared on *Galleria mellonella* larvae. All stages of the life cycle are described and illustrated. Proposed new synonymy includes *Neoaplectana leucaniae* Hoy, 1954 a synonym of *N. bibionis* Bovien, 1937 and *Neoaplectana affinis* Bovien, 1937 a synonym of *N. menozzi* Travassos, 1932.

INTRODUCTION

With the increasing awareness of the potential dangers of persistent insecticides, and with a ban on the use of DDT on pastures, considerable interest has developed in New Zealand in the control of insects by means of natural parasites.

Species of the nematode genus *Neoaplectana* Steiner, 1929 have effectively controlled certain insect species (2) by means of a lethal symbiotic bacterium transmitted by their free-living infective larvae (8, 7, 40). The author is presently studying their potential against pasture pests.

In New Zealand, infective larvae of *Neoaplectana* have been isolated from soil samples from a wide range of localities including sparsely vegetated high country, rain forests, agricultural soils, and sand dunes. A population isolated from the pasture pest *Graphognatus leucoloma* (Boheman) was identified as *Neoaplectana bibionis* Bovien, 1937.

The life cycle of *N. bibionis* was worked out by Bovien (3) and has become generally accepted as basic for the genus (14, 15, 16, 18, 19, 20, 21, 23, 27, 30, 31, 33, 34, 38). The descriptions and text of the original paper (Bovien, 1937) have been misinterpreted because a detailed description of some of the life stages was lacking. This has led to disagreements on facets of the life cycle. Therefore a redescription was undertaken

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of the species, including a detailed study of the life cycle, and the results are presented here.

MATERIALS AND METHODS

Neoaplectana bibionis isolated from infected white fringed weevil larvae (*Graphognatus leucoloma* (Boheman)) collected from pastures near Auckland was propagated in greater waxmoth larvae (*Galleria mellonella* (L)) on damp filter paper in petri dishes at room temperature. The infective juveniles penetrated and killed the host within 48 hours of inoculation and fed and developed on the decomposing body contents. After 10 to 14 days, large numbers of infective larvae (L3) emerged from the cadaver. These were collected either on water agar or in a water trap (9, 42) and could be kept alive and infective for several months in constantly aerated spring water or in 0.1% formalin at 5–10 C. For studies of the mode of entry and the morphology of the various stages of the life cycle, nematodes were extracted individually from the head, tail section, and remainder of the body of the greater waxmoth larvae, in 50% Ringer's solution, at 2-hour intervals, on each of the first 2 days after inoculation. This was repeated at least six times. Active penetration through mouth, anus, and spiracles was studied as described earlier (43).

LIFE CYCLE

The seven distinct stages of the life cycle of *Neoaplectana* species are: the egg, four larval stages, and the two sexes of the adult. The four larval stages are morphologically distinct, and since they are not always produced sequentially, they are, strictly speaking, instars. Here they are referred to as L1–L4. Salient features that separate them are as follows:

L1: The first larval stage, characterised by the undeveloped genital tract, distinct lips with labial papillae, funnel-shaped stoma, and absence of lateral fields. Young L1 are transparent but become dark in appearance from accumulated reserves.

L2: The second larval stage, characterised by the narrow, almost closed stoma, absence of transverse annulation and lateral fields, and persistence of the cuticle during development of the cuticle of the next stage.

L3: The free-living resistant infective third stage, characterised by its slender appearance, transverse annulation of the cuticle, nine lines in the lateral field, distinct deirids and phasmids, almost closed stoma, and the presence of bacteria in the cadiac region of the intestine.

Female L4: The fourth stage or pre-adult female, characterised by its swollen appearance, absence of lines in the lateral fields, advanced development of the genital tract and vulval primordium, and rather short, proximally wide, dorsally often convex, sharply pointed tail.

Male L4: The fourth larval stage or pre-adult male, characterised by its resemblance to the female, the advanced development of the spicule primordia and the short, proximally wide, dorsally often concave tail with narrow terminus.

The life cycle of *N. bibionis* as it takes place in a natural host is schematically presented in Fig. 1. The free-living infective third-stage larva (L3) has a life span that may vary from only a few days to several months in soil. In 0.1% formalin at 5 C it has survived for several years. Penetration takes place through the mouth and anus of the host. Attraction by the mouth seems weak. Of hundreds of L3 applied near the host's head, only a few reached the mouth, and only a small percentage of these penetrated. Penetration through the anus was not observed, although inferred, since

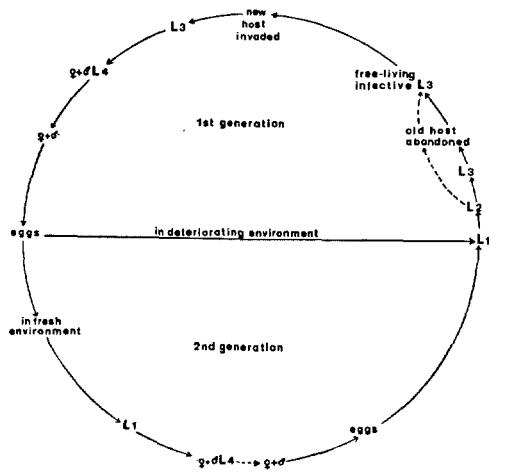

FIG. 1. Schematic presentation of life cycle of *Neoaplectana bibionis* in natural host (solid lines, usual two cycles; broken lines, alternative pathway).

within 2 hours of inoculation the hind part of the body cavity of the host contained infective larvae. Penetration through the spiracles was not established. After entering the host the infective larvae penetrate the wall of the digestive system and enter the body cavity. Some accumulate in the head capsule of the host. They develop considerably before they moult. The development process starts immediately after the body cavity is reached: the stoma gradually opens and becomes functional; the oesophagus, including the basal bulb, expands; and the excretory glands increase in size and become distinct, with three nuclei clearly discernible. The bacterial pouch below the basal bulb swells, and gradually moves posteriad. At about one body width from the basal bulb the contents are released and the bacteria flow into the open lumen of the nematode intestine. The larvae usually grow very little in length but double their width. Within 24 hours they develop into L4.

The L4 doubles in width with little increase in length. Some specimens grow very wide, as if their last moult is delayed. The genital tract develops substantially.

Young females and males are present in the host about 2½ days after inoculation. Initially both sexes are of equal size, but in a suitable environment a reproducing female develops a body several times its original length whereas males increase mainly in width. Females feed and live for about 5 days. Males may or may not feed, but live as long as the females. Early developing young females lay eggs; in later developing and older females most eggs develop in the female, and *endotokia matricida* results.

All eggs develop into L1. In a fresh host, with a low population density no L2 and L3 develop but L1 develop rapidly and directly into L4. When the population density increases, L2 develop that give rise to resistant infective L3.

The L2 has a closed stoma and does not feed. It grows thinner, the oesophagus is reduced, the lumen of the intestine is closed, and as the annulated cuticle of the L3 develops it gradually retracts from the thick L2 cuticle. The nematode then increases in length, ruptures the L2 cuticle (usually just below the head), and becomes a slender resistant infective stage that leaves the old host to start a free-living existence.

In *Galleria* larvae one or two generations may take place, depending on the number of infective larvae originally invading.

REDESCRIPTION OF *NEOAPLECTANA BIBIONIS* BOVIEN, 1937

L1 (Fig. 2A–F): Young transparent L1 feed actively and soon accumulate reserves, giving them a dark appearance. Cuticle smooth. Lateral field absent. Deirids, amphids and phasmids not observed. Lip region slightly set off, with six lips, each with distinct papilla. Lips initially light and transparent, gradually becoming more dense. Stoma triangular in face view. Cheilostom changing from transparent in young to partially reinforced in older animals. Protostom-telostom funnel-shaped, triradiate in face view. Oesophagus with triradiate lumen. Isthmus distinct. Nerve ring around middle of isthmus. Basal bulb distinct, with weak valve. Cardia present. Hemizonid obscure. Excretory pore present, inconspicuous. Excretory glands slightly displace basal bulb dorsally, three nuclei discernible. Intestine an open tube with nucleated cells that gradually become dark with accumulated reserves. Anus distinct, posterior lip somewhat enlarged. Tail conical, with sharply pointed terminus. Body length ranges from 250–700 μm.

L2 (Fig. 2G–O): Cuticle smooth. Lateral field absent; as a result, deirids and phasmids cannot be located with certainty. Lip region with six lips, not set off, each with distinct papillae often directed sideways. Cheilostom closed. Lumen protostom-telostom and oesophagus triradiate. Isthmus distinct, surrounded by nerve ring. Excretory pore indistinct; distinct only late in this stage. Excretory glands large, with three distinct nuclei displacing basal bulb dorsally. Cardia present. Intestine dark with reserves, lumen closed. Anus distinct, with enlarged posterior lip. Tail gradually tapering to slightly drawn-out terminus. Body length approximately 700 μm. If the L2 has developed under unfavourable conditions it can be considerably smaller.

L3, the infective stage (Fig. 3A–I): (n = 20). L = 880 μm (range 750–950); width = 25 μm (22–27); cheilostom length = 2 μm

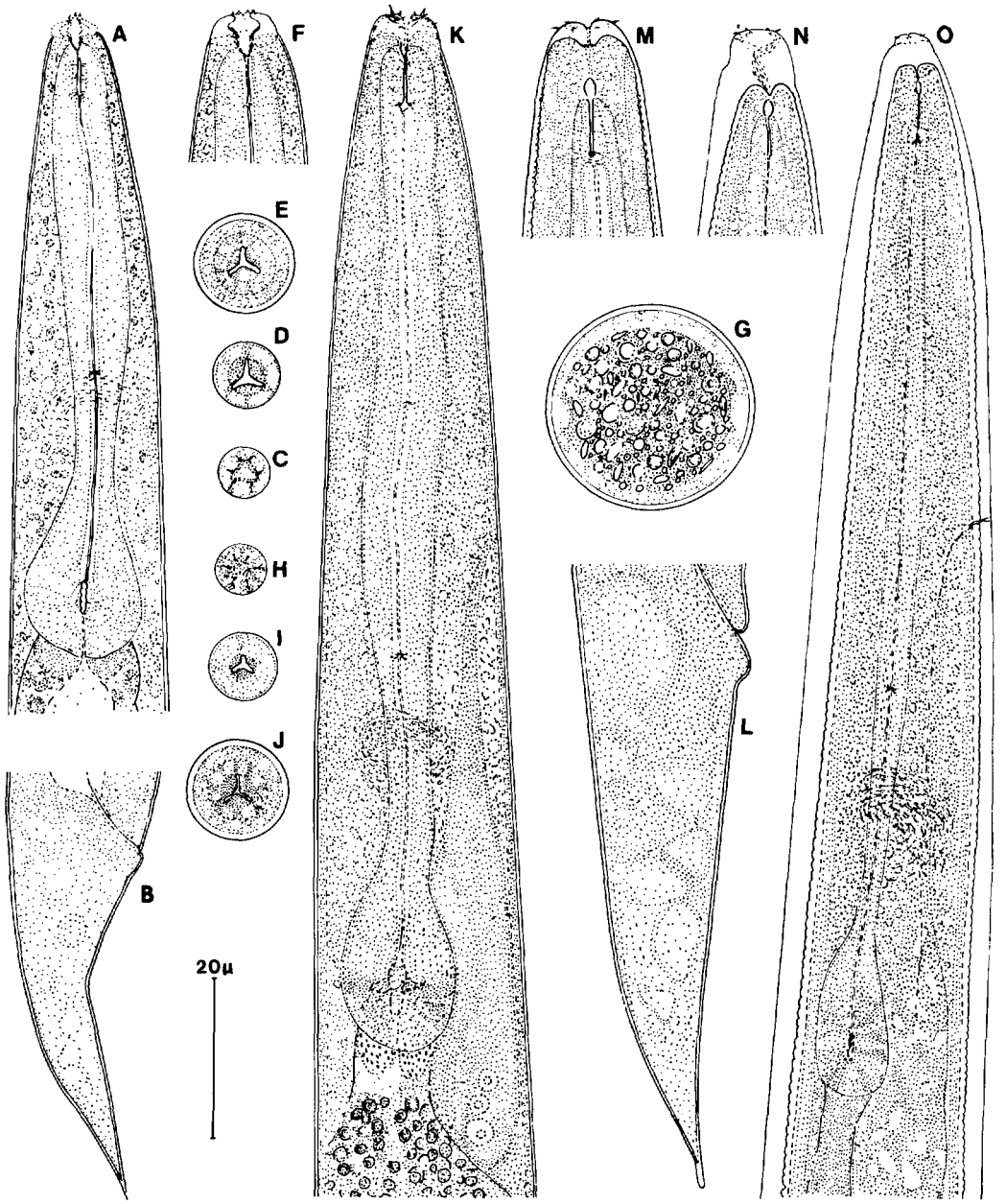

FIG. 2. *Neoaplectana bibionis.* A–F) First larval stage: A) Oesophageal region. B) Tail. C) Face view. D) Cross-section protostom-telostom. E) Cross-section anterior oesophagus. F) Cephalic region late L1. G–L) Second larval stage. G) Cross-section midbody. H) Face view. I) Cross-section proto-telostom. J) Cross-section anterior oesophagus. K) Oesophageal region. L) Tail. M–O) Cephalic region late L2 and developing L3 inside cuticle of L2. M) Late L2. N) Late L2 casting cuticular lining of stoma. O) Developing L3 immediately before moulting.

(1.5–2.5); stoma (closed) length = 13.2 µm (11.5–15.5); lip region width = 6.1 µm (5.0–7.0); oesophagus length = 135 µm (115–150); basal bulb width = 10.8 µm (9–12); length of bacterial pouch in the intestine = 29 µm (18–37); distance of bacterial pouch from base of oesophagus = 9.5 µm (3–16); gonad length = 190 µm (130–230); rectum length = 25.3 µm (20–32); tail length = 83 µm (71–92); H = 32 µm (23–41); the distance from the anterior end to the excretory pore = 62 µm (53–67), to the nerve ring =

100 μm (89–108), to the deirids and hemizonid = 116 μm (105–124), and to the hemizonion = 176 μm (145–193); body width at level of basal bulb = 23 μm (21–25); body width at level of anus = 15.6 μm (15–17); distance of phasmids from tail terminus = 52 μm (45–67).

Body slender, tapering regularly from base of oesophagus to anterior end and from anal area to tail terminus. Cuticle with indistinct transverse annulation, most clearly visible in early development when still surrounded by L2 cuticle, and in late development when growth resumes in new host. Lateral field distinct, with eight ridges resulting in nine lateral lines, starting short distance from head, terminating on tail, the upper and lower submarginal ridges reduced. Deirids at level of hemizonid, small, with distinct subcutaneous supporting structure. Phasmids small, on posterior half of non-hyaline portion of tail. Lip region smooth, generally not set off. Cheilostom a narrow pore in face view surrounded by six labial papillae, six indistinct cephalic papillae, and two amphids. Protostom-telostom triangular in shape anteriorly, changing to triradiate posteriorly. Oesophagus long and narrow, with triradiate lumen, occupying 1/3–1/4 body-width, distinctly narrower at level of nerve ring, terminating in valved bulb. Nerve ring distinct. Excretory pore distinct, located at level of mid oesophagus. Excretory duct long, usually distinct; sclerotised valve joining it to large, ventral excretory glands that displace basal bulb and anterior end of intestine. Hemizonid at level of basal bulb. Cardia present. A sharply delineated pouch of bacteria enclosed by intestinal wall immediately below cardia. Hemizonion variable in position, usually at level of posterior end of bacterial pouch. Lumen of intestine narrow or closed. Intestinal wall dark with globules. Genital tract well developed. Rectum long and narrow. Anus distinct. Tail conical with pointed terminus.

L4, the pre-adult (Fig. 3J–L): Cuticle smooth, lateral lines absent. Lip truncate with six slightly elevated labial papillae and six small cephalic papillae. Amphids indistinct. Cheilostom triangular in face view, lined with inconspicuous sclerotisation, about as long as wide. Protostom-telostom sclerotised, triradiate, funnel-shaped. Oesophagus wide, occupying about half the body width, procorpus distinctly swollen, enhancing the narrow isthmus, lumen triradiate. Nerve ring immediately anterior to basal bulb. Basal bulb pyriform, with valve. Excretory pore at level of middle of oesophagus, distinct, open; excretory glands large, displacing basal bulb dorsally, nuclei distinct. Hemizonid at level of anterior part of basal bulb. Presence of deirids difficult to establish. Cardia present. Lumen of intestine wide. Intestinal wall nucleated. Development of reflexed genital tract well advanced. In late female L4, vulva primordium conspicuous; in late male L4, spicule primordium distinct. Rectum narrow. Anus distinct, with slightly enlarged posterior anal lip. Tail considerably shorter than in L3, in female L4 turning dorsad, and in male L4 more or less straight, terminating in fine tip. Except for the primordia of the sex organs and the tail shape there are no characters to distinguish male L4 from female L4. Body length is 750–950 μm.

Female (Fig. 4A–F): Because of the great variability in female length within the species morphometrics of the female are not considered of diagnostic significance.

Cuticle smooth, lateral lines absent. Lip region initially resembles pre-adult, in larger animals labial papillae increase in size, and lip region and stoma widen, occasionally recessed below the level of the cephalic region. Lip region with six lips, each with one labial papilla at its tip and a cephalic papilla at its base. Amphids obscure. Cheilostom as long as wide with thick non-sclerotised walls. Protostom-telostom funnel-shaped, with tooth-like structure on each arm of triradiate anterior end projecting into stoma. Oesophagus short in relation to body size, similar to L4. Basal bulb pyriform, large compared with other stages, small in relation to body size. Cardia present. Excretory pore at level of procorpus. Excretory glands displacing anterior part of intestine dorsally, nuclei arranged as in L4. Genital tract paired, reflexed. Uterus and spermatheca located ventrally between vulva and flexure, ovaries located dorsally beyond flexure, terminating ventrally in opposite body half. Vulva lips protruding with distinct epiptygma. Rectum and anus distinct. Posterior anal lip usually conspicuously enlarged. Tail tapering rapidly

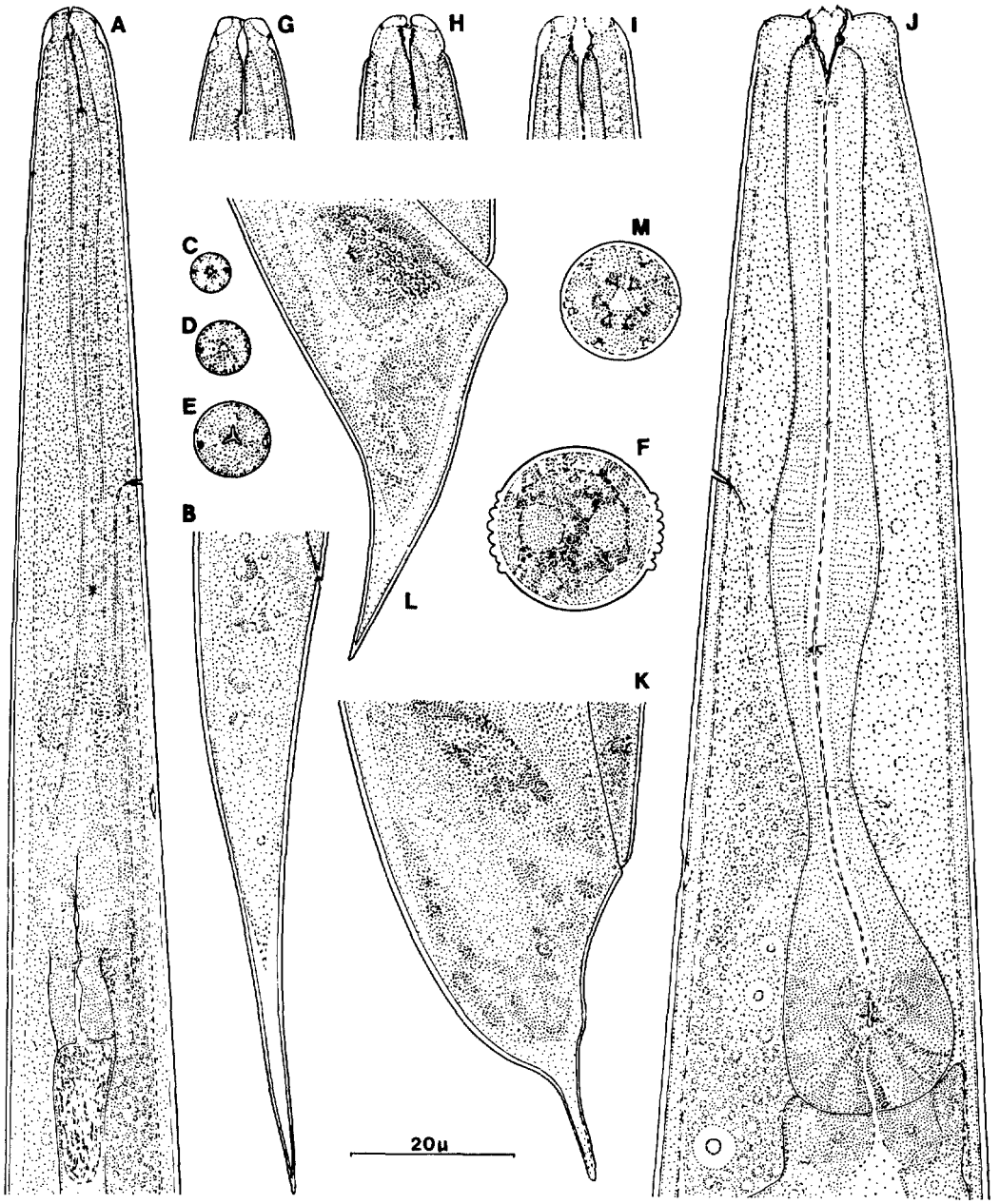

FIG. 3. *Neoaplectana bibionis.* A–I) Third larval stage. A–F) Free-living infective stage. A) Oesophageal region. B) Tail. C) Face view. D) Cross-section protostom-telostom. E) Cross-section anterior oesophagus. F) Cross-section midbody. G–I) Cephalic region parasitic stage. G) Soon after penetration. H–I) Progressive development after feeding has started. J–M) Pre-adult. J) Oesophageal region. K) Male tail L4. L) Female tail L4. M) Face view.

to rounded body end with mucronated terminus. Maximum body length observed: 7 mm.

Male (Fig. 4G–K): (n = 10). L = 934 µm (850–1000); greatest width = 75 µm (60–90); oesophagus length = 130 µm (110–150); basal bulb width = 28 µm (25–32); body width at level of basal bulb = 50 µm (40–65); distance of excretory pore from anterior end = 75 µm (65–85); testis length = 575 µm (475–675); spicules length = 62 µm (60–65); gubernaculum length = 40 µm (35–45); tail length without mucro = 27 µm (22–32); mucro length = 10 µm (8–15).

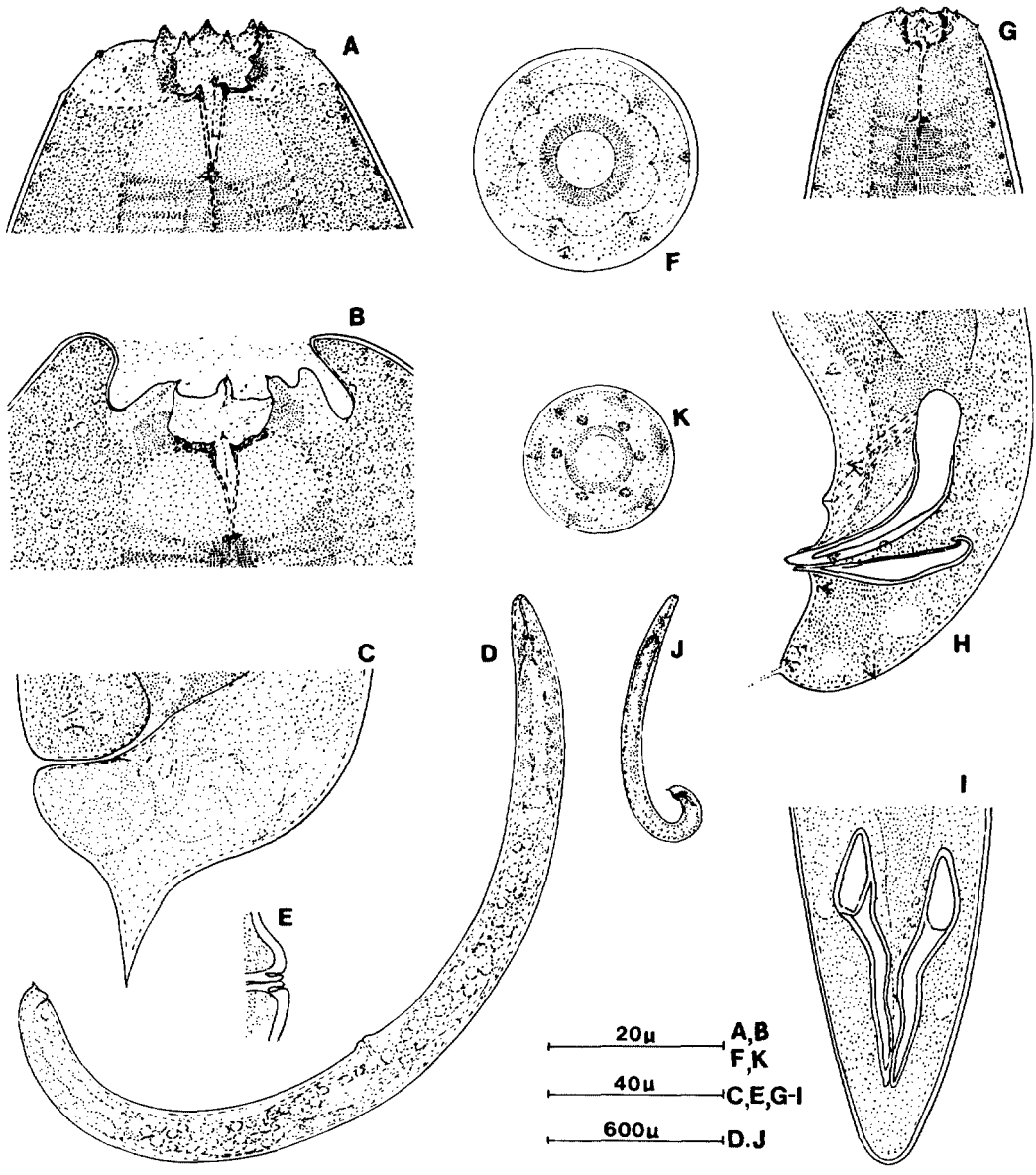

FIG. 4. *Neoaplectana bibionis*. A–F) Female. A) Cephalic region. B) Cephalic region retracted. C) Tail. D) Female whole. E) Vulval region. F) Face view. G–K) Male. G) Cephalic region. H) Tail, lateral aspect. I) Tail, dorso-ventral aspect. J) Male whole. K) Face view.

Cuticle smooth, no lateral lines. Lip region and stoma as in pre-adult except labial papillae slightly larger. Oesophagus similar to L4 except excretory glands located below basal bulb, not displacing it. Excretory pore distinct, wide open, located at level of procorpus. Hemizonid at level of base of oesophagus or further posteriad. Cardia large. Testis reflexed. Spicules heavy, yellow in colour, with large manubrium. Gubernaculum yellow, narrow distally, proximal end usually bent anteriorly and about half maximum-width wide. One large ventral pre-anal papilla and eleven pairs of genital papillae located as follows: seven in subventral line from anterior of spicule heads to just past anus (two most posterior of these usually adanal and postanal); two subterminal (usually subventral); one subdorsal (on distal half of tail); and one

lateral (at level of or slightly anterior to anus). Tail tapering to rounded body terminus with fine mucro. Bursa absent.

Differential diagnosis: The population described was identified as *N. bibionis* since it shares with that species: larvae of intermediate length (650–1000 μm); males with weakly curved yellow spicules with large manubrium and without distal hooks; a spindle-shaped gubernaculum; a spicate tail terminus; and the basic number and distribution of male papillae. It differs from the type species *N. glaseri* Steiner, 1929 (32), by the shorter infective larvae (in *N. glaseri* over 1000 μm) and the absence of hooks on the distal ends of the spicules. It differs from *N. menozzii* Travassos, 1932 (35), by the longer infective larvae (in *N. menozzii* averaging 450 μm) and straighter spicules and longer manubrium, and from *N. feltiae* Filipjev, 1934 (10), by the straighter spicules (the unusual arrangement and large number of genital papillae reported for the males of *N. menozzii* and *N. feltiae* is probably incorrect).

The material studied resembles type material as well as material from the type locality of *N. leucaniae* Hoy, 1954, the only *Neoaplectana* species described from New Zealand. Since the material from the type locality interbreeds readily with the *N. bibionis* population described here, *N. leucaniae* is considered a new synonym of *N. bibionis.*

N. affinis Bovien, 1937, of which Bovien (3) stated: ". . . . the species may appear to be identical with *N. menozzii*", resembles *N. menozzii* in its strongly curved spicules with short rounded manubrium, and the absence of a spicate male tail, and is here considered a new synonym of this species.

In New Zealand, developing stages of *Neoaplectana* species have been isolated from a wide range of arthropods: pupae of the noctuid *Leucania acostis* Meyrick; adults and larvae of the melolonthids *Costelytra zealandica* (White), *Odontria communis* Given, *O. nitidula* Broun, *O. autumnalis* Given, *O. striata* (White), *Pyronota festiva* (Fabricius), *P. inconstans* Brookes; larvae of the hepialid *Wiseana cervinata* Walker, the noctuid *Persectania ewingi* Westwood, the pyralid *Orocrambus simplex* (as *Crambus simplex* Meyrick), and the scarab *Pericoptus* sp. (16); larvae of the curculionids *Cecyropa discors* Broun (Dale, pers. comms) and *Graphognatus leucoloma* (Boheman); larvae and adults of the mites *Lancetoppia mahunkae* Hammer and *Oppia arcualis* Berlese; and several unidentified small arthropods. At least three different *Neoaplectana* species are represented.

DISCUSSION

Species of the genus *Neoaplectana* are not well defined (29, 39). Specific characters of seven species have been described but of the remaining twelve species only characters of the genus are known (36). Stanuszek (29) recognised three species and suggested that additional species should not be recognised until it has been established that they do not interbreed with those three and that they have been described from material reared in a common host (31). A revision of the genus is obviously overdue, although identification of the present population as *N. bibionis* is considered correct.

Bovien (3), in his original description of the species, indicated that the infective stage, the L3, remains for some time enclosed in the cuticle of the preceding stage. This agrees with my observations. Unfortunately this presence of an ensheathing L2 cuticle, which was assumed to be shed in the new host (16) either in the intestine (22, 28) or in the body cavity (27), has been confused by later workers (16, 18, 19, 21, 22, 27, 30, 38) as a condition prerequisite to infection. This is incorrect. The L2 cuticle is retained for an extended period only in the old host. With the possible exception of *N. carpocapsae* Weiser, 1955 (37), the L2 cuticle is usually shed before the L3 becomes free-living and it is always shed before the L3 becomes infective. The L2 cuticle is retained somewhat longer when the development of the L2 is prematurely initiated and insufficient reserves have accumulated for the development of a full-sized L3. Over-estimating the significance of the L2 cuticle of the infectives has also led to misinterpretation of results of anatomical studies (17, 23).

Passive entry of the host can be directly observed in mosquito larvae (4, 24, 41). By this mode of entry many infective larvae are digested (6). Active entry through the mouth and anus of *Galleria* larvae is less

easy to observe but is more effective. Ten infective larvae per 100 g of soil suffice to kill *Galleria* (1). Entry through mouth and anus is probably the common mode of entry in nature although entry through the spiracles has been reported (11, 39). It was originally assumed that, after reaching the alimentary tract of the host, the nematodes awaited the death of the host (3) before reproducing (5, 13). Later it was discovered that this is atypical of neoaplectanids (25), and that gut penetration usually follows immediately after the alimentary tract is reached (22). Once the infectives have reached the body cavity of the host they release into the host lethal bacteria that were contained in a sharply delineated pouch in the cardiac region of the intestine (26). The presence and symbiotic nature of these bacteria in the infectives was suggested by Bovien (3) and later confirmed (7, 8, 40). In the body cavity of the host the L3 develop and change considerably before they moult.

Bovien (3) described the pre-adult only summarily. He did not report cephalic papillae. He noted the spicule mother cells but the associated ventrally curved dorsally concave tail of the male L4 was not reported. The most obvious character that separates male from female pre-adults is the ventrally located uninterrupted section of the genital tract between mid-body and anus.

Bovien (3) described the adults most extensively and accurately. Only cephalic papillae in the female were overlooked, and in the male the shape of the proximal end of the gubernaculum was described as a hook, which in this study was not present consistently. Observed here also were the mucronated male tail and the presence of small forms that are disproportionately thick (3, 16). The more nutrient available, the larger the females grow. According to Schmiege (27) large hosts produce large females. Females grow longest when they develop individually in a host in the absence of a male.

The early observation that young are born (12, 14, 16) is incorrect. Egg packets are dispersed by the female which moves intensively while she lays them (31).

Differences in appearance and size between the L1 that hatches from the egg and the rather long dark larvae developing from them made Bovien (3) decide that they belong to different stages. Differences in size, color, and appearance between extremes of a stage, however, are typical for the genus, and the dark form is still L1. This late L1 has a thin cuticle only fragments of which remain upon moulting.

The further development of the L1 in the genus *Neoaplectana* is similar to the development of the L1 in the genus *Heterorhabditis* Poinar, 1976 (43). In a favourable environment they develop directly into the actively feeding pre-adult (L4) without going through a second or third larval stage. In an unfavourable environment the L1 give rise to L2.

The late L2 has a thick cuticle in which the L3 can be observed developing to an advanced stage. The L3 does not become infective until the L2 cuticle is cast. In most species this takes place before the old host is abandoned.

Bovien (3) assumed that the infective third larval stage of *N. bibionis* has a disseminating function since he observed them clinging to the outside of a host. In this study too, infectives were observed accumulating around insects in the soil, occasionally in such numbers that they form a "nematode wool" that could be observed with the naked eye. Similarly attached to adult insects, they may be transported over long distances, particularly in a humid environment. This phenomenon may explain the wide distribution of this species in New Zealand and, possibly, the world.

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