

# A new species of *Neoaplectana* Steiner, 1929 (Nematoda : Steinernematidae) from Córdoba, Argentina

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## SUMMARY

*Neoaplectana rara* n. sp., an entomophagous nematode parasite of *Heliothis* sp. (Lepidoptera : Noctuidae) from the Province of Córdoba, Argentina, is described. This species is distinguished from other members of the genus by the absence of cephalic papillae, by the shape of vulvar lips, spicules and gubernaculum, and by the number of genital papillae.

## RÉSUMÉ

Une nouvelle espèce du genre *Neoaplectana* Steiner, 1929 (Nematoda : Steinernematidae) provenant de Córdoba, Argentine

*Neoaplectana rara* n. sp. nématode entomophage parasite d'*Heliothis* sp. (Lepidoptera : Noctuidae) provenant de la Province de Córdoba, Argentine, est décrit et figuré. Il se différencie des autres espèces du genre par l'absence de papilles céphaliques, la forme des lèvres vulvaires, des spicules et du gubernaculum, et par le nombre des papilles génitales.

During a nematological survey of entomophagous nematodes at Córdoba, Argentina (Doucet, 1982), a nematode belonging to an undescribed species of the genus, *Neoaplectana* Steiner, 1929 (Steinernematidae) was observed. It is described and figured below.

## Materials and methods

Nematodes were obtained from soil samples from maize fields in the area of Rio Cuarto, Province of Córdoba. In the laboratory, larval wax-moth, *Galleria mellonella* L. (Lepidoptera : Galleriidae) were used to isolate the nematodes from soil according to a new technique (Doucet, in press) and for reproduction of the nematode (Poinar, 1975). The infective juveniles penetrated this host, killed it within 24-48 h and fed and developed on the decomposing body contents.

The nematodes were extracted from the wax-moth larvae in Ringer's solution on each of the first five days after exposure, then every other day until the 15th day to study the biology and the morphology of the various stages. The nematodes were fixed in warm 7 % formaldehyde solution, then transferred to a glycerin 15 % alcohol solution, slowly dehydrated and mounted in anhydrous glycerin.

For examination on the scanning microscope, the nematodes were fixed as mentioned above, after washing in Ringer's solution. The nematodes were dehydrated for one hour in ethanol/water mixtures of 50/50, 70/30, 80/20, 90/10, 95/5 and in pure ethanol. Then, they were dried at critical point and coated with gold. The SEM used was a Philips 501 B at 15 KV accelerating voltage.

## *Neoaplectana rara* n. sp

(Figs 1, 2, 3)

## DIMENSIONS

*Females, first generation* (n = 20) : Total length (L) = 5.2-10.4 mm (7.39 ± 1.4); greatest width = 132-216 µm (169 ± 23.3); length of stoma = 6.5-12 µm (9 ± 1.47); width of stoma = 9-12.5 µm (11 ± 1.39); length, head to base of oesophagus = 205-250 µm (219 ± 14), to excretory pore = 65-113 µm (93 ± 11.2), to nerve ring = 132-183 µm (163 ± 12); V = 52-66 (57 ± 3,1); length of tail = 37-50 µm (44 ± 4,6); width at anus = 65-110 µm (84 ± 13,1); length of mucron = 4-6 µm (5 ± 0,6).

*Females, second generation* (n = 20) : Total length = 3.1-4.1 mm (4 ± 0,28); greatest width = 143-177 µm

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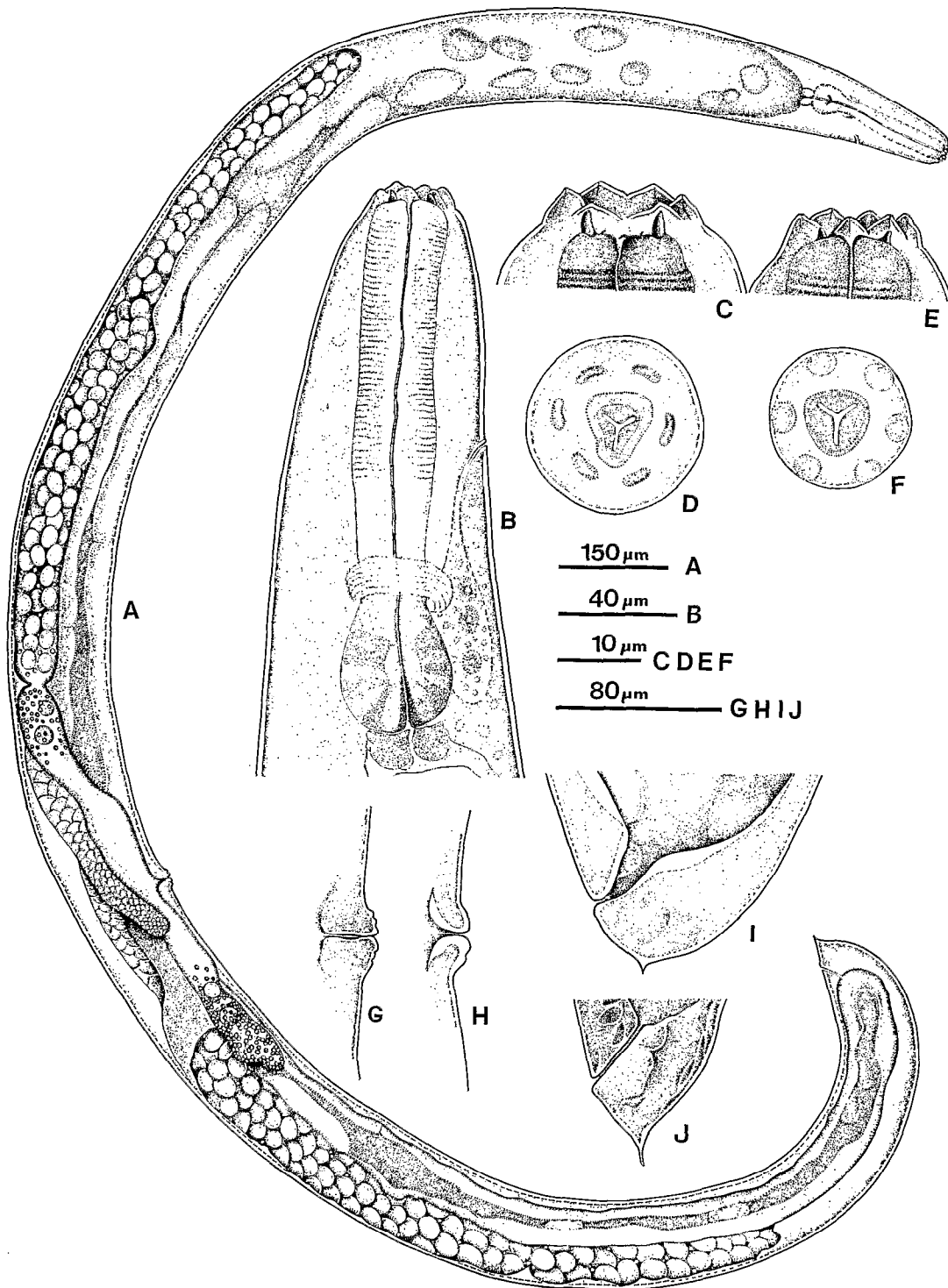


Fig. 1. *Neoplectana rara* n. sp. Female, first generation. C : anterior region (lateral view); D : lip region (en face view); H : vulvar region; I : tail (lateral view). Female, second generation. A : animal *in toto*; B : oesophageal region; E : anterior region (lateral view); F : lip region (en face view); G : vulvar region; J : tail (lateral view).

(156 ± 11.1); length of stoma = 4-5.5 µm (5 ± 0.32); width of stoma = 10-12.5 µm (12 ± 0.7); length, head to base of oesophagus = 177-202 µm (190 ± 7.6), to excretory pore = 75-95 µm (88 ± 5.7), to nerve ring = 138-157 µm (150 ± 5.8); V = 55-62 (58 ± 1.9); length of tail = 38-51 µm (46 ± 3.3); width at anus = 53-73 µm (62 ± 6.1); length of mucron = 7.5-12.5 µm (10 ± 1.2).

*Males, first generation* (n = 20) : Total length = 1.3-1.7 mm (1.6 ± 1.1); greatest width = 100-142 µm (123 ± 9.8); length of stoma = 2-5 µm (3 ± 0.7); width of stoma = 3-5 µm (4.5 ± 0.7); length, head to base of oesophagus = 125-150 µm (135 ± 9.1), to excretory pore = 55-77 µm (67 ± 8.2), to nerve ring = 91-120 µm (103 ± 7.9); length from origin to flexure of testis = 175-543 µm (347 ± 88); length of tail = 35-52 µm (43 ± 4.7); width at anus = 40-57 µm (50 ± 4.2); length of spicule = 42-52 µm (47.5 ± 3.3); width of spicule = 5.5-9 µm (7 ± 0.8); length of gubernaculum = 23-38 µm (34 ± 7.1); length of mucron = 2-5 µm (3 ± 0.8).

*Males, second generation* (n = 20) : Total length = 0.7-1.1 mm (0.8 ± 0.2); greatest width = 45-63 µm (53 ± 6.5); length of stoma = 2-3 µm (2.5 ± 0.3); width of stoma = 5-7 µm (6 ± 0.5); length, head to base of oesophagus = 106-120 µm (114 ± 4.1), to excretory pore = 47-70 µm (60 ± 6.4), to nerve ring = 75-92 µm (86 ± 4.9); length from origin to flexure of testis = 163-242 µm (150 ± 20.5); length of tail = 32-42 µm (38.5 ± 2.5); width at anus = 30-38 µm (33 ± 2.6); length of spicule = 32-40 µm (36 ± 2.5); width of spicule = 5-6.5 µm (6 ± 0.4); length of gubernaculum = 25-30 µm (27 ± 1.4); length of mucron = 3-6 µm (5 ± 0.7).

*Juvenile 3rd stage* (n = 20) : Total length = 443-563 µm (499 ± 30.7); greatest width = 19-26 µm (22 ± 1.7); length, head to base of oesophagus = 89-105 µm (96 ± 4.5); to excretory pore = 34-40 µm (37 ± 1.7); to nerve ring = 60-72 µm (66.5 ± 2.8), to hemizonid = 70-79 µm (74 ± 2.7); length of tail = 43-55 µm (49 ± 2.8).

*Holotype* (female, first generation) : Total length = 7.4 mm; greatest width = 181 µm; length of stoma = 12 µm; width of stoma = 10 µm; length, head to base of oesophagus = 223 µm, to excretory pore = 83 µm, to nerve ring = 160 µm; V = 56; length of tail = 41 µm; width at anus = 93 µm; length of mucron = 4 µm.

#### DESCRIPTION

*Female, first generation* : Cuticle smooth, lateral lines and phasmids not observed. Head truncate to slightly

rounded; lips united, mouth opening subtriangular; one circle of six labial papillae with a kidney-shaped contour, lacking cephalic papillae; amphids not observed. Stoma reduced; cheilostome and oesophagus triangular in *en face* view, lined with inconspicuous sclerotization; oesophageal tissue close to mouth opening, reaching to the base of the vestibule; prorhabdions not evident; cheilorhabdions represented as sclerotized areas; meso-, meta- and telorhabdions vestigial. Oesophagus muscular, short and wide in relation to length of body, the anterior portion slightly expanded, extending into a low non-valvulated metacorpus, narrowing at level of isthmus and terminating in a basal bulb, which is valvated and pyriform; base of oesophagus not inserted into anterior portion of intestine; cardia well developed. Nerve ring surrounding isthmus just anterior to basal bulb. Excretory pore circular, anterior to level of nerve ring. Genital tract amphidelphic; opposed reflexed ovaries in dorsal position; ovaries lead to a well developed glandular oviduct; spermatheca and uterus located ventrally between vagina and flexure; vagina short with muscular walls; vulva a transverse slit, generally protruding from the body surface; vulvar lips smooth. Eggs commonly develop and hatch inside the reproductive system; "endotokia matricida" is frequent. Tail bluntly conical to dome-shaped with a short spine on the tip. Rectum and anus distinct.

*Female, second generation* : Similar to first generation, differing from them by the following characters : smaller size, labial papillae with rounded contour, vulvar lips with transverse creases, conic tail with a well developed mucron. Pigmy forms never found.

*Male, first generation* : Cuticle, lip region and oesophageal region similar to those of first generation females. Testis single and reflexed, consisting of a germinal growth zone leading into a seminal vesicle. *Vas deferens* with inconspicuous muscular walls. Spicules paired, heavy, symmetrical, curved, with ribs, and bearing a rounded inconspicuous arch in their ventral surface. Capitulum round, head and lamina bearing ridges in edges and surface. A thin velum is present. Distal lips of spicules usually obtuse with membranous appearance. Gubernaculum variable in shape ranging from flattened to bow-shaped in lateral view, with a small proximal digital portion upturned or not. Tail with a complement of nineteen genital papillae : seven ventrolateral pairs, two lateral pairs and a single ventral. Four pairs of ventrolaterals are preanal, one pair adanal and two pairs post-anal; the two lateral pairs are postanal and the ventral papilla is pre-anal. Tail conical, tapering to a fine mucro. Bursa absent.

*Male, second generation* : Similar to first generation; differ from them in the following characters : smaller size, labial papillae with rounded contour, thinner spicules, tail with a well developed mucron.

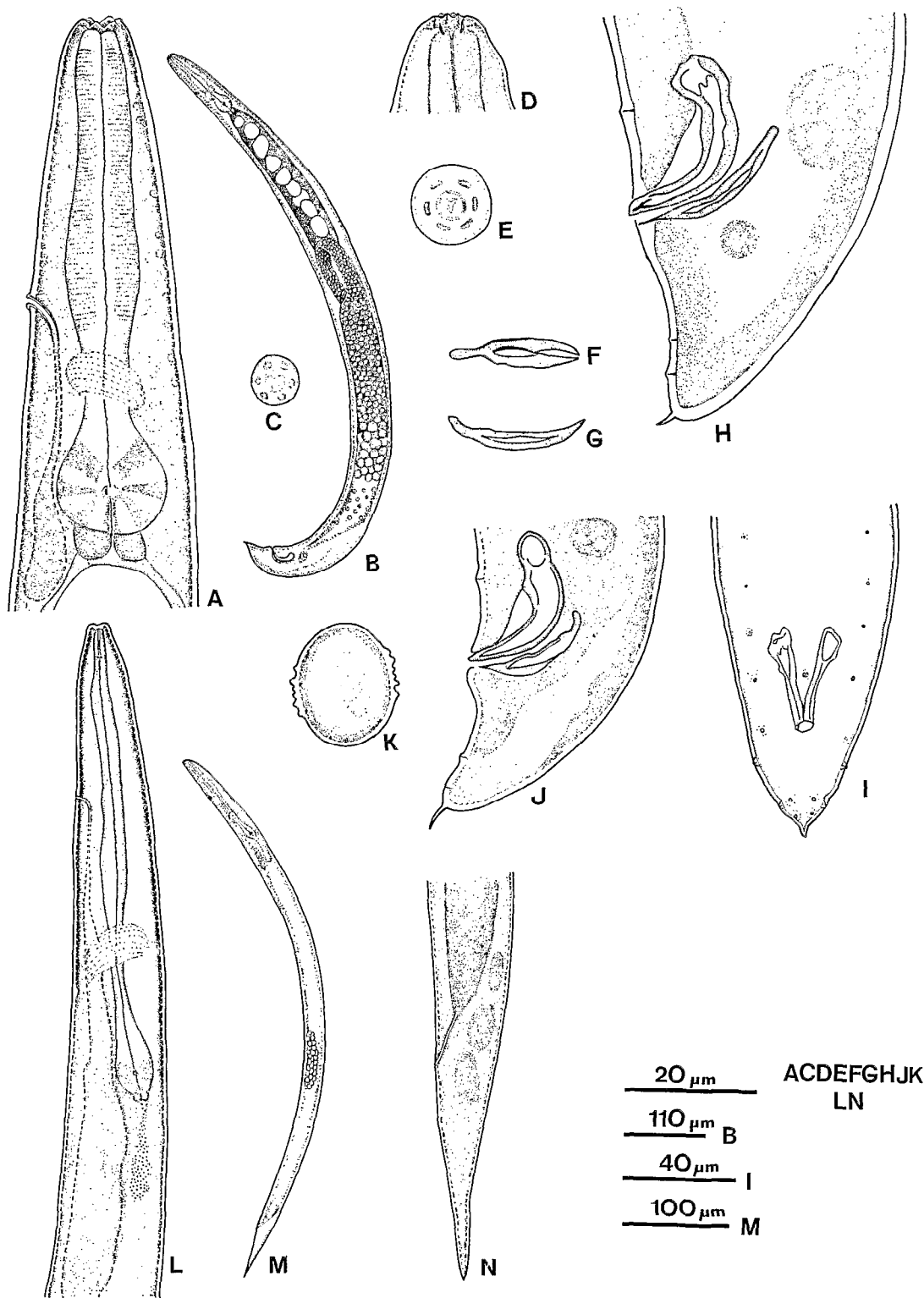


Fig. 2. *Neoaplectana rara* n. sp. Male, first generation. D : anterior region (lateral view); E : lip region (en face view); F-G : gubernaculum (ventral and lateral view); H-I : tail (lateral and ventral view). Male, second generation. A : oesophageal region; B : animal *in toto*; C : lip region (en face view); J : tail (lateral view). Third infective stage. K : cross section through middle body; L : oesophageal region; M : animal *in toto*; N : tail (lateral view).

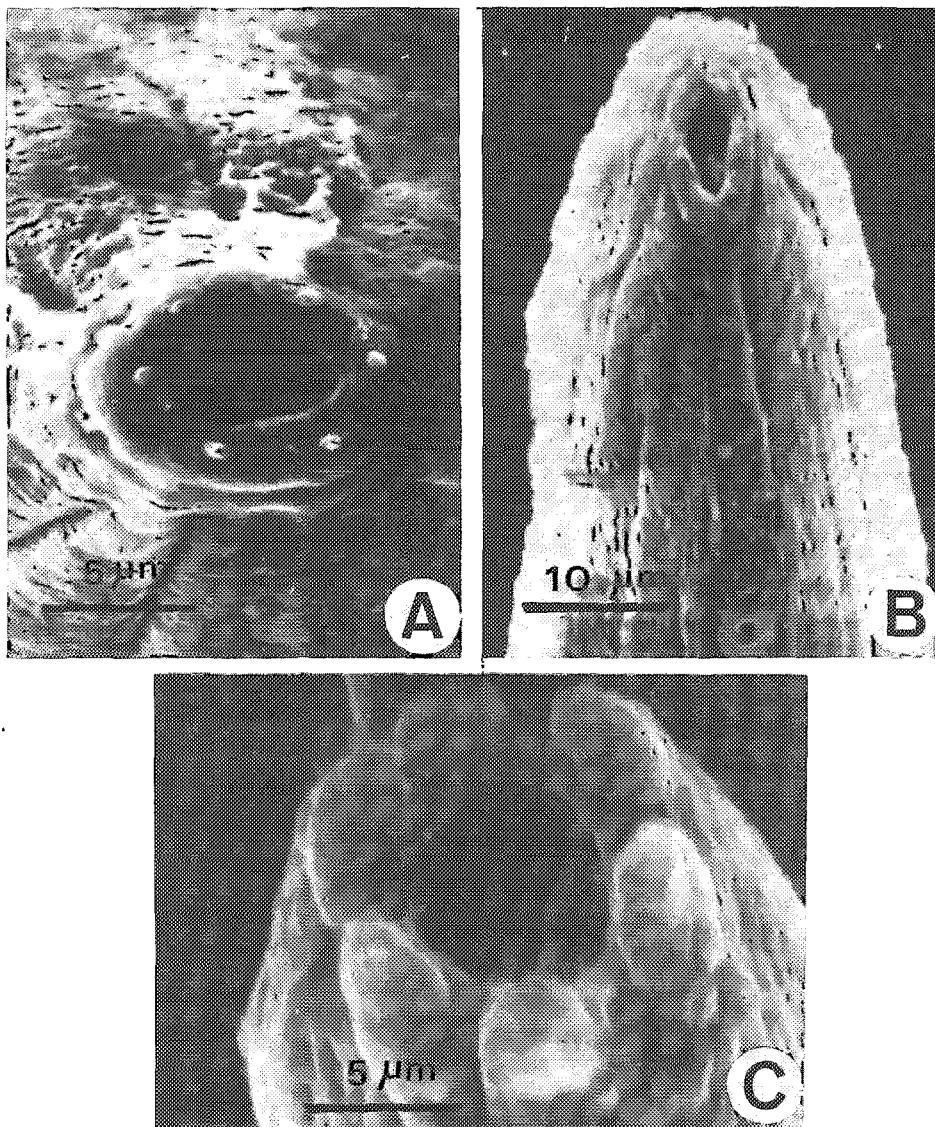


Fig. 3. *Neoplectana rara* n. sp. (SEM pictures). Female, first generation. A : lip region (en face view); B : anterior region (ventral view). Female second generation. C : lip region (en face view).

*Third infective juvenile stage* : Body slender, tapering regularly from base of oesophagus to anterior end and from anus to terminus. Cuticle with faint transverse annulation. Lateral field distinct, lined by six ridges. Lip region smooth, generally not set off. Mouth and anal openings closed. Oesophagus long and narrow, distinctly narrower at level of nerve ring, terminating in a valvated basal bulb displaced dorsally. Cardia present. A sharply delineated pouch of bacteria enclosed by intestinal wall, posterior to cardia. Lumen of intestine

narrow and closed. Rectum long and narrow. Anus distinct. Excretory pore at level of first half of oesophagus. Excretory duct long, usually distinct; ventral excretory glands displace the basal bulb, pouch of bacteria and anterior end of intestine dorsally. Nerve ring distinct, anterior to basal bulb. Hemizonid at level of basal bulb. Genital primordium well developed, in the middle of the posterior half of the body. Tail conical with pointed terminus. Exceptionally the third juvenile stage remains within the cuticle of the second stage.

#### TYPE HOSTS AND HABITAT

In the body cavity of *Heliothis* sp. (Lepidoptera : Noctuidae).

#### TYPE LOCALITY

Rio Cuarto, Province of Córdoba, Argentina.

#### TYPE SPECIMENS

*Holotype* : female, on slide RAX/171-6, deposited in the Centro de Zoología Aplicada, Universidad Nacional de Córdoba, Argentina. *Paratypes* : specimens distributed as follows : five females and five males of first generation in Station de Recherches sur les Nématodes, INRA, Antibes, France and in University of California, Davis, Ca, USA. Remaining specimens deposited in the Centro de Zoología Aplicada.

#### DIAGNOSIS AND RELATIONSHIPS

*Neoaplectana rara* n. sp. can be separated from all other species of the genus by the absence of cephalic papillae. In the morphology of the spicules *N. rara* n. sp. is close to *N. carpocapsae* Weiser, 1955, *N. feltiae* Filipjev, 1934 and *N. georgia* Kakulia & Veremchuk, 1956. It differs from the first in the number of genital papillae in males of the first generation (19 vs 23), in the position of the basal bulb with respect to the anterior end of the intestine (oesophagus not inserted in intestine vs oesophagus inserted in it) and in the cuticle of the third infective juvenile stage (absence vs presence of second-stage juvenile cuticle). It differs from the other two species in the length of the third infective juvenile stage (443-563  $\mu\text{m}$  vs 750-850  $\mu\text{m}$  and 780-820  $\mu\text{m}$  respectively).

*N. rara* n. sp. is close to *N. menozzi* Travassos, 1932 in general morphology of females and males of the second generation and in length of third juvenile stage, but differs from it in shape of tail-tip (presence vs absence of mucron), in length of spicules (32-40  $\mu\text{m}$  vs 54-56  $\mu\text{m}$ ) and in length of gubernaculum (25-30  $\mu\text{m}$  vs 40  $\mu\text{m}$ ).

#### Life cycle

The free-living infective juvenile can survive for three months in 0.1 % formaldehyde and in soil at 4° and it takes on a C shape.

Penetration takes place through the mouth and anus of the host; penetration through the spiracles was not established. It was inferred to take place through the anus and mouth, since within 12 hours of inoculation the hind and anterior part of intestine contained infective juveniles. After entering the host, the infective juveniles go through the wall of the digestive system and enter the body cavity. They develop considerably after

penetration. The development process begins immediately after the body cavity is attained; the changes are evident in that the stoma opens gradually, the oesophagus and bulb expand and the lumen of the intestine is open. The excretory glands grow in size and become functional. Within 24 hours the juveniles grow in width and develop into L4. The preadult stage is semitransparent and particularly active, and the genital tract develops substantially. The adult stage quickly follows at 48 hours. Young females and males are initially equal in size but females develop to several times their original length whereas males increase mainly in width.

For egg development, the female must be fertilized. As gestation proceeds, a mixture of eggs in different stages of cleavage and first, second and third instar juveniles may be seen within the uterus.

Some juveniles are usually born 24-30 hours after the females reach maturity, others remain within the uterus and "endotokia matricida" results.

Females feed and live for about 4-5 days and males can survive 6-7 days. Normally all eggs develop to J1 then to J2, L3, L4 and adults of the second generation; however some J2 develop into resistant infective L3 when the population density is great.

The second generation takes place after five days; females and males are shorter than the first generation. Development continues to resistant infective J3. The second instar juveniles formed cease to grow, the lumen of the alimentary canal is closed, the oesophagus is reduced, ecdysis takes place and the old cuticle is retained as a loose sheath.

About twelve days after inoculation the adults are dead and the carcass of the insect quickly becomes a sac packed with nematodes. At the same time the J3 usually ruptures the J2 cuticle (at 15-20  $\mu\text{m}$  below the head) and leaves the old host to start a free living existence.

The infective-stage juvenile contains cells of bacteria in the ventricular portion of the intestine. These cells are liberated when the juveniles invade the host haemocoel. The bacteria turn the freshly killed insect juvenile to a reddish colour in two days and then the normal coloration returns. The bacteria invade the host tissue completely within 24 hours after penetration.

About 24 hours after the L3 penetration, the insect dies.

#### Remarks

*Neoaplectana* Steiner, 1929 was defined by the presence of six labial and six cephalic papillae (Steiner, 1929). After the observations of four cephalic papillae in the species *N. glaseri* Steiner, 1929; *N. feltiae* Filipjev, 1934 and *N. bibionis* Bovien, 1937 the character of six cephalic papillae has been widely disputed (Mráček & Weiser, 1979; Weiser, Mráček & Gerdin, 1981, Wouts et al., 1982; Poinar, 1984).

*N. rara* n. sp. is characterized by the presence of six labial papillae and the absence of cephalic papillae. This characteristic would exclude it from the genus *Neoaplectana* but the rest of its characters are the same as those that define this genus. Until other species of the genus are re-examined as to the number and disposition of cephalic papillae, the author prefers to include the species described in this paper in the genus *Neoaplectana*. Eventually the number of cephalic papillae should be considered a specific and not a generic character, as proposed by Steiner (1929).

Species of *Neoaplectana* are not well defined (Stanusek, 1974a). The specific characters are not established and in most of the descriptions of species it is not clear whether the description is based on the first or the second generation (Turco, Thorne & Hopkins, 1971; Poinar, 1979). A distinct dimorphism (Stanusek, 1974b; Mráček, 1977; Poinar, 1978) is frequently found in Steinernematid nematodes between the first and the second generation, which was observed also in this study. The author considers that this phenomenon should be taken into account to reach a good definition of the species of this genus.

The life cycle of *N. rara* n. sp. was studied and accords with that generally accepted as basic for the genus (Hoy, 1954; Poinar & Himsworth, 1967; Stanusek, 1974b; Nickle, 1977; Poinar, 1979). The further development of the J1 is similar to that in other *Neoaplectana* species, except as described in *N. bibionis* (Wouts, 1980). Although the late J2 has a thick cuticle in which the L3 can be observed developing to an advanced stage; the latter does not become infective until the J2 cuticle is cast.

The ability of this nematode to develop in laboratory conditions is encouraging and makes it a possible candidate for biological control in a program for pest management.

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