Neoaplectana intermedia n. sp. (Steinernematidae : Nematoda) from South Carolina

George O. POINAR, Jr.

Department of Entomological Sciences, University of California Berkeley, CA 94720, USA.

SUMMARY

Neoaplectana intermedia n. sp. (Steinernematidae : Nematoda) is described from South Carolina. Morphological, hybridization and DNA studies support the distinctness of *N. intermedia* n. sp. in comparison with populations of *N. carpocapsae, N. glaseri* and *N. bibionis.* Diagnostic characters include the length of the infective stage juveniles, the shape of the spicules and gubernaculum and absence of a tail projection in the male. The infective juveniles of *N. intermedia* n. sp. carry a population of the symbiotic bacterium *Xenorhabdus nematophilus* in an intestinal "pouch". These bacteria differ from those associated with the other three above-mentioned species of *Neoaplectana.* The life cycle is typically neoaplectanid with the infection initiated by infective stage juveniles and one or more parasitic generations occuring inside the insect. Monoxenic cultures of *N. intermedia* n. sp. have been established on artificial media.

RÉSUMÉ

Neoaplectana intermedia *n. sp. (Steinernematidae : Nematoda) provenant de Caroline du Sud)*

Neoaplectana intermedia n. sp. (Steinernematidae : Nematoda) provenant de Caroline du Sud est décrit. Les études concernant la morphologie, les croisements et le DNA prouvent l'originalité de *N. intermedia* n. sp. vis à vis des populations de *N. carpocapsae, N. glaseri* et *N. bibionis.* Les caractères diagnostiques concernent la longueur des juvéniles infestants, la forme des spicules et du gubernaculum et l'absence de projection terminale sur la queue du mâle. Les juvéniles infestants de *N. intermedia* n. sp. transportent une bactérie symbionte, *Xenorhabdus nematophilus,* dans une « poche » intestinale. Cette bactérie diffère de celles associées aux trois autres espèces de *Neoaplectana* citées plus haut. Le cycle biologique est typiquement néoaplectanide, l'infestation débutant par les juvéniles (stade infestant) et une ou plusieurs générations lui succédant à l'intérieur de l'insecte. Des élevages monoxéniques de *N. intermedia* n. sp. ont pu être établis sur milieux artificiels.

The genus *Neoaplectana* was erected by Steiner in 1929 with the type species *N. glaseri.* Synopses of the genus were presented by Turco, Thomas and Hopkins (1971) and Poinar (1979). Recently the genus was proposed to be synonymized with *Steinernema* (Wouts *et al.,* 1982), however, the present author does not agree with this action (Poinar, 1984).

Although some sixteen species have been described in the genus *Neoaplectana,* it is clear that for species description, studies on interspecific variability and hybridization are needed to confirm distinctness. DNA analysis is another tool that indicates differences between species. Of the described *Neoaplectana* species these tests have been conducted with only those few species which are represented today as living populations. Thus, there are only three well described and distinct species in the genus. These are *N. carpocapsae* Weiser, *N. bibionis* Bovien and *N. glaseri* Steiner. The present paper adds a fourth species to this list. This neoaplectanid was recovered from infected wax moth larvae (*Galleria mellonella* L.) that had been placed in soil as "trap insects" by Charlie Creighton in Charleston, South Carolina.

Materials and methods

Infective juveniles were sent to the author by Charlie Creighton. They were used to infect last instar wax moth larvae and the progeny were used for the present description. First generation adults were collected four days after infection and second generation adults six or seven days after infection. These stages, as well as infective juveniles which left the cadaver, were heat killed, fixed in 3 % TAF and processed to glycerin for measurements. Infective stages were also measured and examined just after being heat killed. For controlled matings, infective stages of the present species, along with those of *N. glaseri* (Florida strain), *N. carpocapsae* (42 strain) and *N. bibionis* (KL strain), were placed in hanging drops of insect *(Galleria)* blood. After reaching the adult or pre-adult stage, males and females of the same and different species were placed together in separate blood drops and observed for ten days.

The symbiotic bacteria were isolated from infective stage juveniles of the new neoaplectanid using the blood drop method (Poinar, 1966). Monoxenic cultures of this neoaplectanid were established on dog food slants by

taking females from the above mentioned blood drop and transferring them to sterile media.

Populations of the new species, together with populations of *N. glaseri, N. bibionis* and *N. carpocapsae* were sent to John Curran for selection and comparison of restriction fragment length differences of genomic DNA. Digestion of genomic DNA with restriction endonucleases generates a unique set of different sized DNA restriction fragments dependent upon the base sequence of the genome. The size distribution of these restriction fragments is unique to the genotype and can be analyzed by agarose gel electrophoresis (Curran, Baillie & Webster, 1985).

Results

Interbreeding experiments between the new neoaplectanid and the three previously described species, *N. glaseri, N. carpocapsae* and *N. bibionis* were negative while controls using the same species were positive.

These results, together with morphological and nucleic acid studies, indicated that the South Carolina neoaplectanid is a new species and it is described below. In the quantitative portion of the description, measurements (all in microns unless otherwise indicated) are given of both the larger first generation and smaller second generations adults.

Neoaplectana intermedia n. sp.
(Figs. 1 & 2)

Steinernematidae Chitwood & Chitwood (1937) 1950, *Neoaplectana* Steiner, 1929.

MEASUREMENTS

See Table 1 (females) and Table 2 (males).

DESCRIPTION

Adults forms. Cuticle smooth, head rounded, not offset from rest of body; six lips united at base but distinct at tips. Each lip bearing a papillum. Four cephalic papillae occur further back on the head, located in sub-medial positions. Amphids distinct, located behind lateral papillae in same circle as cephalic papillae. Stoma partially collapsed; pharyngeal collar absent, pharynx extending nearly to mouth opening. Cheilorhabdions represented by a thick ring of sclerotized material just beneath the lips. Below this is another sclerotized ring that represents the prorhabdions. Meso-, meta- and telorhabdions are vestigal and would occur in the collapsed area of the stoma. Pharynx muscular with a cylindrical procorpal area followed by a slightly swollen nonvalvated metacorpus. Below this is the isthmus followed by a basal bulb containing a modified valve lined with refractive ridges. The nerve ring often surrounded the anterior portion of the basal bulb. The excretory pore opening varied, but tended to be posterially located in general, this tendency being most pronounced in the first generation males. Lateral fields and phasmids inconspicuous. The sexes are separate and reproduction is by amphimixis.

Females : Amphidelphic with opposed reflexed ovaries. Vulva a transverse slit, usually protruding slightly from the body surface. The vagina is short and leads into paired uteri. Eggs deposited initially, but they later hatch inside the females and the juveniles bore their way out. First generation adults larger than those of the second generation and first generation females with a wide tail with a rounded wedge-like projection on the tip. Tail of second generation females straight and pointed, sometimes with a slight postanal ventral swelling. Pigmy forms occurred in some instances.

Males : With a single reflexed testis. In the seminal vesicle could be found secondary spermatocytes dividing into spermatids and spermatids shedding their residual bodies. The spicules are paired and strongly curved. The tips are blunt. Sometimes, the upper section of the lamina protrudes slightly over the lower portion giving a bifid appearance. The capitulum is broad and blunt, rarely pointed; a ventral arch is usually prominent on the calomus yet can be reduced or almost absent. From this arch, the velum extends obliquely nearly to the tip of the lamina. The gubernaculum is boat shaped in lateral view, with the proximal portion curved upwards and enclosing the spicule shafts. In ventral view, the gubernaculum is forked, with a membraneous structure running from one side of the fork to the other.

There are a complement of twenty three genital papillae (eleven pairs and a single ventral adanal) that are consistently present but vary somewhat in position. Three pairs are consistently found on the tail tip, and three pairs occur in the region posterior to the gubernaculum (Fig. 1J). Above the cloaca in a lateral ventral position extend a row of five pairs up the body. Just above the cloaca is a single ventral papillae that is usually quite consistent in its position. The tail is occupied by a pair of genital papillae. The ventral portion of the tail is usually concave. This character seems to be fairly consistent although the degree of concavity varies somewhat. A thin flap of sclerotized cuticle occurs over the cloaca and serves to close the cloacal opening when the spicules are in a resting position. The cloacal opening is slit like and on both sides are a pair of small lateral papillae-like structures (Fig. 1K). Whether these are innervated could not be determined but if so they could function as sensory organs to locate the vulva.

Infective stage juveniles (third stage enclosed in second stage cuticle) : These are narrower than the corresponding parasitic juveniles. The mouth and anus are closed and the pharynx and intestine are collapsed (Fig. 2a).

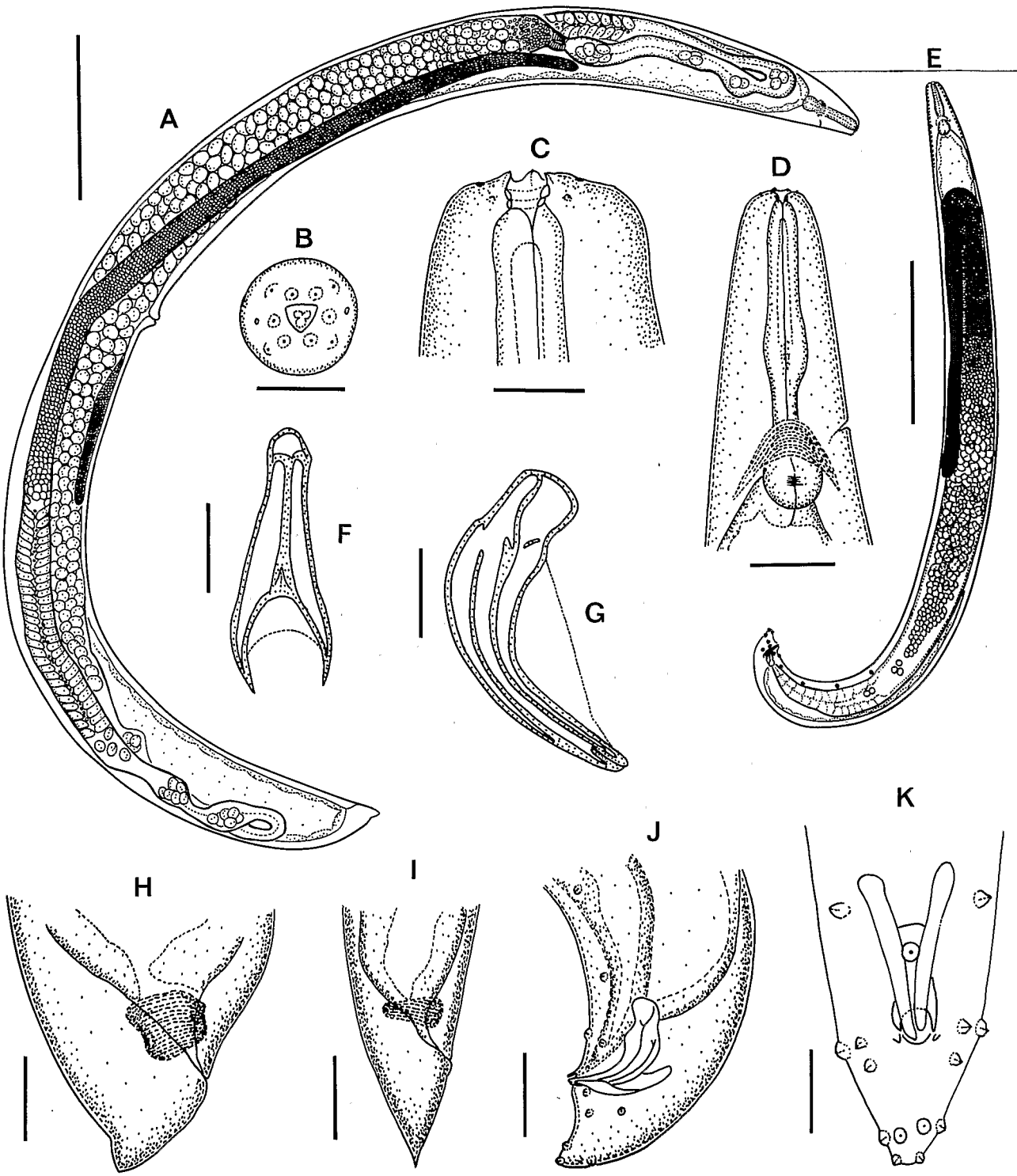

Fig. 1. *Neoaplectana intermedia* n. sp. C, H : first generation female. C : head (ventrad). H : tail. A, I : second generation female. A : animal *in toto;* I : tail. B, D, E, F, G, J, K : male. B : *en face* view of anterior end; D : pharyngeal region; E : animal *in toto;* F : gubernaculum (ventrad); G : spicule (laterad); J : tail (laterad); K : tail (ventrad).

(bars representent : A, E = 400 µm; B, C, F, G = 20 µm; D, H, I, J = 50 µm; K = 30 µm)

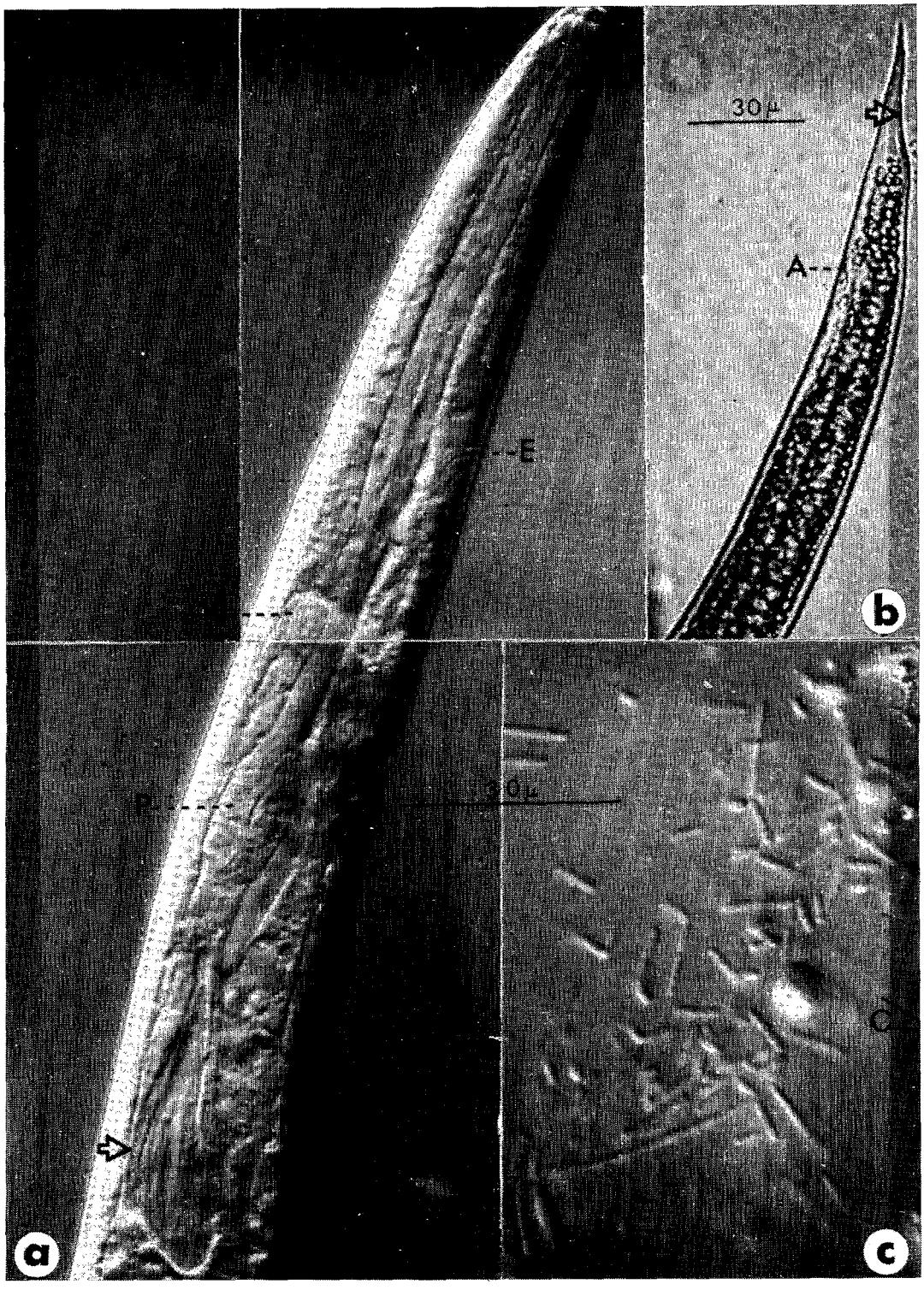

Fig. 2. *Neoaplectana intermedia* n. sp. Infective stage juvenile. a. Lateral view of anterior portion showing excretory pore (E) basal bulb of pharynx (P) nerve ring (N) and bacterial pouch containing cells of *Xenorhabdus nematophilus* (arrow). b. Tail showing characteristic dorsal constriction (arrow : A = anus). c. Cells of *X. nematophilus* in the blood of a *Galleria mellonella* larva invaded by *N. intermedia.*

Table 1

Comparative measurements of first and second generation females of *Neoaplectana intermedia* n. sp. (n = 10)

Character	*First generation*		*Second generation*	
	X	*Range*	*X*	*Range*
Total length (mm)	5.2	3.3-6.4	2.2	1.9-2.6
Greatest width	236.0	170.0-277.0	111.5	94.5-126.0
Length stoma	6.8	4.8-8.0	3.7	3.2-4.8
Width stoma	8.0	6.4-9.6	5.7	4.8-6.4
Length head to excretory pore (EP)	132.0	108.0-143.0	117.0	105.0-127.0
Length head to nerve ring	158.0	101.0-181.0	146.0	133.0-159.0
Length head to pharynx base (Ph. B.)	218.0	193.0-244.0	189.0	174.0-203.0
Length tail	71.0	60.0-89.0	62.0	57.0-70.0
Width of anus	101.0	73.0-127.0	51.0	41.0-63.0
Percentage vulva (V)	53.0	50.0-57.0	55.0	52.0-57.0
Ratio : head to EP/head to Ph. B.		0.51-0.61		0.56-0.66

Table 2

Comparative measurements of first and second generation males of *Neoaplectana intermedia* n. sp. (n = 10)

Character	*First generation*		*Second generation*	
	X	*Range*	*X*	*Range*
Total length (mm)	2.4	1.6-3.0	1.2	1.1-1.3
Greatest width	168.0	113.0-207.0	71.0	63.0-95.0
Length stoma	3.8	3.2-4.8	2.7	1.6-3.2
Width stoma	5.6	4.8-8.0	5.0	4.8-6.4
Length head to excretory pore (EP)	137.0	114.0-155.0	97.0	85.0-108.0
Length head to nerve ring	146.0	120.0-168.0	128.0	117.0-136.0
Length head to pharynx base (Ph. B.)	190.0	155.0-209.0	164.0	158.0-171.0
Length reflexion of testis	625.0	283.0-1 008.0	225.0	183.0-290.0
Length of tail	54.2	44.8-59.2	41.0	35.0-45.0
Width at cloaca	77.0	53.0-88.0	49.0	43.0-56.0
Length spicules	93.0	80.0-106.0	65.0	59.0-69.0
Width spicules	19.0	12.8-26.0	13.0	10.0-19.0
Length gubernaculum	62.0	48.0-96.0	36.0	34.0-40.0
Width gubernaculum	11.0	8.0-17.0	7.3	5.6-8.0
Ratio : head to EP/head to Ph. B.		0.67-0.80		0.54-0.63

The tail is constricted, specially on the dorsal side (Fig. 2b). The lateral field is composed of six to eight incisures depending on the region of the body observed. In the anterior portion of the intestine of the infective stages of *N. intermedia* n. sp. occurs a pouch containing cells of the symbiotic bacteria *Xenorhabdus nematophilus* (Fig. 2a). The length of the infective stages (n = 25) was 680 µm (608-800), the greatest width, 28 µm (25-32); distance from the head to the excretory pore 65 µm (61-69); distance from the head to the nerve ring, 92 µm (85-96); distance from the head to the base of the pharynx 121 µm (110-131); length of the tail, 64 µm (53-72); width at anus, 16 µm (13-18) and length of bacterial pouch (27-48 µm). There was no refractile spine in the tail of the infectives.

Bacterial associates

Infective stages of *N. intermedia* n. sp. carry cells of *Xenorhabdus nematophilus* in their intestine and liberate these in the insect host soon after penetration (Fig. 2c). The bacteria have been isolated using the blood drop method (Poinar, 1966) and cultured on nutrient and Tergitol-7 agar plates. The bacteria produce a brownish

cream color on nutrient agar and a greenish blue color on Tergitol-7 agar. They will be described in greater detail in a separate paper.

DNA ANALYSIS

Dr. Curran's characterization of genomic DNA of *N. intermedia, N. bibionis, N. carpocapsae* and *N. glaseri* resulted in bands of restriction fragments that were unique for each species and showed roughly the same amount of distinctness between them. These bands represented multiple copies of respective DNA sequences and the restrictive fragment length differences between such bands were used as diagnostic characters. This technique has already been used to show distinctness among populations of *N. glaseri, N. carpocapsae* and *N. bibionis* (Curran, Baillie & Webster, 1985).

TYPE LOCALITY

Charleston, South Carolina, USA; no type host : recovered from soil using wax moth larvae as bait.

TYPE SPECIMENS

Holotype (male) and allotype (female) deposited in the Nematology collection at the University of California, Davis, California. Paratypes deposited at the Laboratoire des Vers, Muséum national d'Histoire naturelle, Paris.

DIAGNOSIS

Neoaplectana intermedia n. sp. can be separated from *N. carpocapsae, N. bibionis* and *N. glaseri* by several characters. One of these is the length of the infective juveniles which range from 600-800 µm. The lower end of this range does overlap with the upper end of *N. carpocapsae* (438-650 µm) and the upper end of the range overlaps with the lower range of *N. bibionis* (700-1 000 µm), yet on the average, the infectives are larger than those of *N. carpocapsae* and smaller than those of *N. bibionis*. The infectives of *glaseri* range from 864-1448 µm. Also, the tail of the infective stage of *N. intermedia* n. sp. is usually constricted, especially on the dorsal side. A constriction is lacking in infectives of *N. bibionis* and if present occurs on the ventral surface in *N. carpocapsae* and *N. glaseri*.

The male tail of *N. intermedia* n. sp. lacks a projection or spine of any type which separates it from both *N. carpocapsae* and *N. bibionis* (Poinar, 1967; Wouts, 1980). The spicules are generally more curved (a line running parallel with the calomus and lamina forms an angle of 70-90°) than those of *N. carpocapsae, N. bibionis* and *N. glaseri*. Their lack of color (clear) separates them from the yellow spicules of *bibionis*, and their blunt tips separate them from the hooked tip spicules of *N. glaseri* and the pointed spicules of *N. carpocapsae* (Poinar, 1967, 1978). The gubernaculum of *N. intermedia* n. sp. is unusual since the distal portion is bent upwards and encloses the distal portion of the spicules. The male tail of *N. intermdeia* n. sp. has a ventral concavity which is not present or as distinct in the other three neoaplectanids.

Discussion

The strain of *N. intermedia* n. sp. described here is called the SC strain (after South Carolina). The specific name is derived from the length of the infective stages, which are intermediate between those of *N. carpocapsae* and *N. bibionis*. The comparative measurements, which show little overlap between the first and second generation adults of *N. intermedia* n. sp., again point out the great variability found within a single population of neoaplectanids and demonstrate how difficult it is to use quantitative characters in separating adults. Because of this variability, ratios such as the length from the head to the excretory pore divided by the length of the pharynx have been used as diagnostic characters (Poinar, 1979). In the present case, this character was fairly constant in both generations of females (0.51-0.61 *vs* 0.56-0.66) but in the males, there was no overlap (0.67-0.80 and 0.54-0.63). This was due to the extremely posterior position of the excretory pore in the large first generation males. So if ratios are to be used, the generation and sex should be specified.

Each neoaplectanid species has its own particular bacterial "strain" or subspecies (Akhurst, 1983). The population of *Xenorhabdus nematophilus* isolated from *N. intermedia* n. sp. also appears to differ from those originating from previously described neoaplectanids. This bacterium will be described further in a separate study.

Of the previously described neoaplectanids, *N. intermedia* n. sp. most closely resembles *N. affinis* Bovien. Both *N. bibionis* and *N. affinis* were described by Bovien (1937) as parasites of bibionid flies. The characters Bovien used to separate these species were : 1. Males of *N. bibionis* possessed yellow or brownish spicules and gubernacula in contrast to pale grey spicules of *N. affinis*. 2. The spicules of *N. affinis* were somewhat more curved and the manubrium much smaller (not more than 1/5 the length of the total spicule). 3. The gubernaculum of *N. affinis* was more evenly curved and more crescent-shaped without a proximal knob or hook. 4. The infective stages of *N. affinis* were of somewhat shorter size and had à shorter tail than *N. bibionis*. 5. The tip of the tail of the infective larvae of *N. affinis* contained a small spine-like structure which was not present in those of *N. bibionis*. The male tail of *N. affinis* is figured as lacking a spine (in contrast to the figure of *N. bibionis*) by Bovien (1936) however in his text he says in regarding the male tail of *N. affinis* : " The tail may be

more or less blunt or provided with a tip, or not" and regarding the male tail of *N. bibionis*, Bovien (1937) states "a small tip may, or may not be present". Thus, Bovien states that the males of either species may or may not have a mucron on the tip of the tail.

In an attempt to elucidate the relationship between these species, Poinar and Lindhardt (1971) re-isolated neoaplectanid populations from bibionids in Denmark and a description of this material was later presented by Poinar (1979). In this population, males showing variably shaped spicules were encountered and Poinar (1979) concluded that *N. affinis* and *N. bibionis* were intraspecific forms. However, although the diagnostic characters separating the species are slight (no measurements for the infective stage of *N. affinis* were given by Bovien) it now appears that the description presented by Poinar (1979) for *N. bibionis* could fit better with *N. affinis*, since the infectives are smaller (600-780 µm) than the value Bovien gave for *N. bibionis* (700-1 000 µm) and the males rarely had a tail projection.

In this redescription of *N. bibionis* from New Zealand, Wouts (1980) described the males as possessing a tail projection and the infectives ranging from 750-950 µm in length. These two characters, along with the colored (yellow to brown) spicules are diagnostic for *N. bibionis* as it is presently known. Wouts (1980) then synonymized *N. affinis* with *N. menozzii* Travassos. The infective stages of *N. menozzii* range from 400-600 µm in length and the male tail lacks a projection. The size of the infective stages and the anteriorly located excretory pore in the male separate this species from *N. intermedia* n. sp.

N. intermedia n. sp. can be distinguished from *N. affinis* as described by Bovien (1937) and redescribed by Poinar (1979) by the bluntly tipped spicules, more posterior position of the excretory pore and lack of refractive spine in the tail of the infective stages, a character Bovien (1937) claimed was constantly present in *N. affinis*.

The present species can be separated from *N. feltiae* Filipjev, 1934, whose infective stages (750-850 µm) also overlap those of *N. intermedia* n. sp., by the lack of a tail projection on the male. The males of *N. feltiae* possess a relatively long tail projection based on the figures of Filipjev (1934).

The orange-colored spicules and projection on the male tail of *N. georgica* Kakulia & Veremchuk separate it from *N. intermedia* n. sp.

Life history studies indicates that *N. intermedia* n. sp. has a biology comparable to that of the existing species of *Neoaplectana*. Infective stage juveniles enter the hemocoel of insects, liberate their associated bacteria, complete one or two generations, depending on the initial dose and then emerge from the cadaver as infectives. Using the blood drop technique (Poinar, 1966), monoxenic cultures of *N. intermedia* n. sp. have been established on dog food agar. Infective juveniles placed in insect blood drops reached the adult stage in 48 h and produced eggs in 72 h at 20°. The infective stages can be stored at 6-10°.

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