Redescription of *Neoaplectana affinis* Bovien (Rhabditida : Steinernematidae)

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SUMMARY

Neoplectana affinis Bovien is redescribed from material collected by Poinar and Lindhardt in 1971 from infected bibionid flies in Denmark. *N. affinis* is morphologically distinct from previously described members of the genus and can be distinguished from them by the following characters : color and shape of the spicules, presence of a minute tail spine in the male, length of the infective stage and presence of a spine in the tail of the infective stage. Interbreeding tests showed that *N. affinis* and *N. bibionis* represent non-hybridizing populations.

RÉSUMÉ

Redescription de Neoaplectana affinis *Bovien (Rhabditida : Steinernematidae)*

Neoaplectana affinis Bovien est redécrit à partir de matériel récolté par Poinar et Lindhart en 1971 sur des mouches bibionides infestées, au Danemark. *N. affinis* est morphologiquement distinct des espèces décrites antérieurement dans le genre et s'en sépare par les caractères suivants : couleur et forme des spicules, présence d'une petite épine caudale chez le mâle, longueur du corps chez le stade infestant et épine caudale également chez ce dernier. Des essais de croisements ont montré que *N. affinis* et *N. bibionis* constituent des populations incapables de s'hybrider.

Neoaplectana affinis was described in 1937 by Bovien from diseased bibionid fly larvae in Denmark. Since that time it has not been recovered from any other locality but in 1971, *N. affinis* was reisolated from Danish bibionid fly larvae by Poinar and Lindhardt (1971). The material studied by Bovien (1937), and Poinar and Lindhardt (1971) consisted not only of *N. affinis* but also *N. bibionis* Bovien since both nematodes inhabit the same niche as parasites of bibionid flies in Denmark.

Whereas Poinar (1979) earlier had considered the possibility that *N. affinis* and *N. bibionis* represented an example of morphological variation within a single species, further investigations showed that both forms represented distinct, non-interbreeding populations. In order to clarify the distinction between *N. affinis* and other members of the genus, a redescription of *N. affinis* is presented here.

Materials and methods

The population of *N. affinis* used in the present study originated from unidentified bibionid larvae near the city of Skive in Denmark (Poinar & Lindhardt, 1971), a site where Bovien (1937) also collected *N. affinis* from the bibionid fly, *Phila febrilis* (L.) (Bibionidae : Diptera). The nematodes had been maintained through infections of last instar wax moth *(Galleria mellonella)* larvae. In the present study, infection of a fresh group of wax moth larvae was initiated at 21°. First generation adults were collected five days after infection and second generation adults nine days after infection. These stages, as well as infective juveniles which left the cadaver, were heat killed (60°), fixed in 3 % TAF and processed to glycerin for measurements.

For controlled matings, infective stages of the present species, along with those of *N. glaseri* (Florida strain), *N. carpocapsae* (Breton strain), *N. bibionis* (SN strain), and *N. intermedia* (SC strain) were placed in separate hanging drops of wax moth blood. After reaching the pre-adult stage, males and females of the same and different species were placed together in separate blood drops and observed for ten days.

Monoxenic cultures of this neoaplectanid together with its associated bacterium were established on dog flood slants by taking females from blood drops and transferring them to sterile media.

Results

Interbreeding experiments between the *N. affinis* and the four other previously described species were negative while controls using the same species were positive. A comparison of the present isolate with Bovien's original slide preparations of *N. affinis* showed the two populations to be morphologically similar.

In the quantitative portion of the following redescription, measurements (all in µm unless otherwise indicated) are given of both the larger first generation and smaller second generation adults (Tabs 1 & 2).

Table 1

Neoaplectana affinis Bovien
Comparative measurements of first and second generation females (n = 10)

Character	*First generation*		*Second generation*	
	mean	*range*	*mean*	*range*
Total length (mm)	3.1	2.6-3.6	1.7	1.6-1.8
Greatest width	161.0	139.0-208.0	96.0	88.0-107.0
Length stoma	7.4	6.4-8.0	4.0	3.2-4.8
Width stoma	8.2	6.4-9.6	6.0	4.8-6.4
Length head to excretory pore	102.0	82.0-133.0	103.0	93.0-107.0
Length head to nerve ring	131.0	114.0-152.0	111.0	96.0-136.0
Length head to pharynx base	203.0	178.0-228.0	170.0	158.0-179.0
Length tail	53.0	47.0-63.0	55.0	51.0-64.0
Width of anus	73.0	54.0-95.0	39.0	34.0-42.0
Percentage vulva	55.0	50.0-60.0	57.0	54.0-61.0

Table 2

Neoaplectana affinis Bovien
Comparative measurements of first and second generation males (n = 10).

Character	*First generation*		*Second generation*	
	mean	*range*	*mean*	*range*
Total length (mm)	1.8	1.4-2.1	1.3	1.2-1.5
Greatest width	118.0	95.0-164.0	69.0	63.0-76.0
Length stoma	4.2	3.2-6.4	3.4	1.6-6.4
Width stoma	5.1	3.2-6.4	4.5	3.2-9.8
Length head to excretory pore	94.0	82.0-114.0	93.0	86.0-104.0
Length head to nerve ring	105.0	89.0-117.0	119.0	107.0-128.0
Length head to pharynx base	153.0	136.0-174.0	155.0	146.0-168.0
Length reflexion of testis	715.0	485.0-926.0	220.0	189.0-378.0
Length of tail	51.0	45.0-56.0	40.0	30.0-42.0
Width at cloaca	60.0	53.0-72.0	44.0	40.0-50.0
Length spicules	70.0	67.0-86.0	69.0	62.0-72.0
Width spicules	14.0	11.0-19.0	10.0	8.0-11.0
Length gubernaculum	46.0	37.0-56.0	36.0	30.0-42.0
Width gubernaculum	9.6	8.0-10.0	7.0	4.8-8.0

Neoaplectana affinis Bovien, 1937

(Figs 1-2)

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

Measurement

Females : see Table 1.
Males : see Table 2.
Infective stage : ♂3; (n = 15). L = 693 µm (608-880); a = 23 (21-28); b = 5.5 (5.1-6.0); c = 10.5 (9.5-11.5); d* = 0.49 (0.43-0.53); e* = 0.94 (0.74-1.08); max body diam. = 30 µm (28-34); dist. head/excret. pore = 62 µm (51-69); dist. head/nerve ring = 95 µm (88-104); dist. head/pharynx base = 126 µm (115-134); tail length = 66 µm (64-74); anal body diam. = 17 µm (16-19).

Description

Adults : Cuticle smooth, head rounded, not offset from rest of body, six lips united at base but distinct at tips. Each lip bearing a single labial papilla forming an inner circle of six papillae. A second outer circle of four cephalic papillae occur, one at the base of each dorsolateral and ventro-lateral papillae. Paired amphids occur at the base of the lateral papillae. Stoma partially collapsed with the posterior portion engulfed by pharyngeal tissue. Cheilorhabdions represented by a thick ring of sclerotized material lining the fused area of the lips. Below this is a second sclerotized ring that probably represents the prorhabdions. The mesorhabdions are thin walled and partially collapsed. The metarhabdions represented by a small swollen area are vestigal. The pharynx is muscular with a cylindrical procorpal area followed by a slightly swollen nonvalvated metacorpus. Below this is the isthmus followed by a nearly spherical basal bulb containing a reduced valve lined with refractive ridges. In the female the nerve ring is located more towards the center of the isthmus while in the male, the nerve ring usually rested on the anterior portion of the basal bulb. The excretory pore opening was more posteriorly located in males than in females. The lateral fields and phasmids were inconspicuous. The sexes are separate and reproduction is by amphimixis.

Females : Amphidelphic with opposed reflexed ovaries. The vulvar lips protrude from 6-25 µm in the first generation females and 0-5 µm in the second generation females. In addition the first generation females usually have a terminal knob at the base of the tail, measuring from 6-19 µm in length, while the second generation

* d = dist. head / excret. pore divided by dist. head / pharynx base; e = dist. head / excret. pore divided by tail length (see Poinar, 1986).

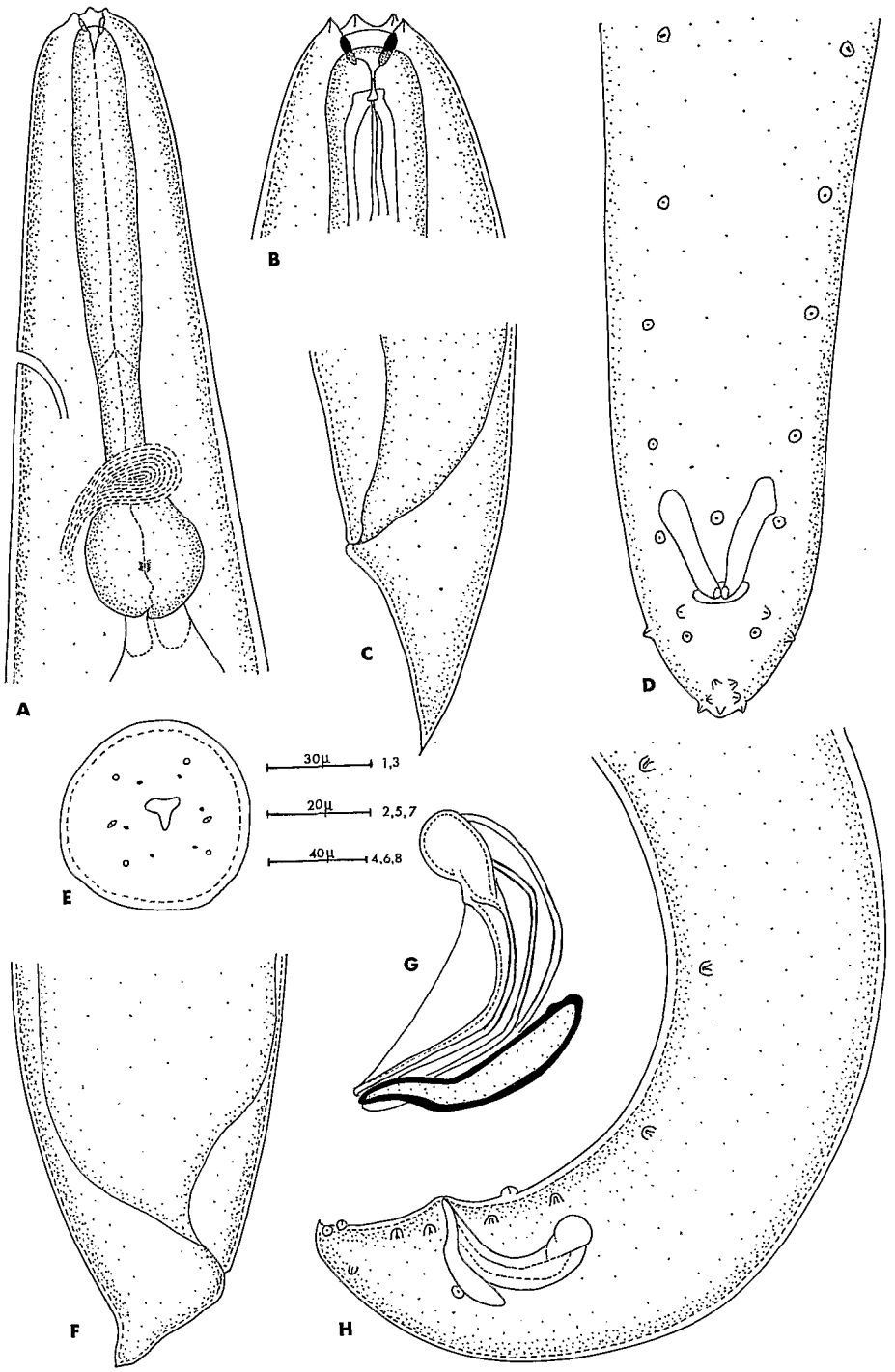

Fig. 1. *Neoaplectana affinis* Bovien, 1937. Adults. A : Lateral view of anterior portion of first generation male; B : Lateral view of lip region of first generation female; E : " En face " view of the first generation female; D : Ventral view of first generation male tail; F : Lateral view of first generation female tail; C : Lateral view of second generation female tail; G : Lateral view of spicules and gubernaculum of first generation male; H : Lateral view of first generation male tail.

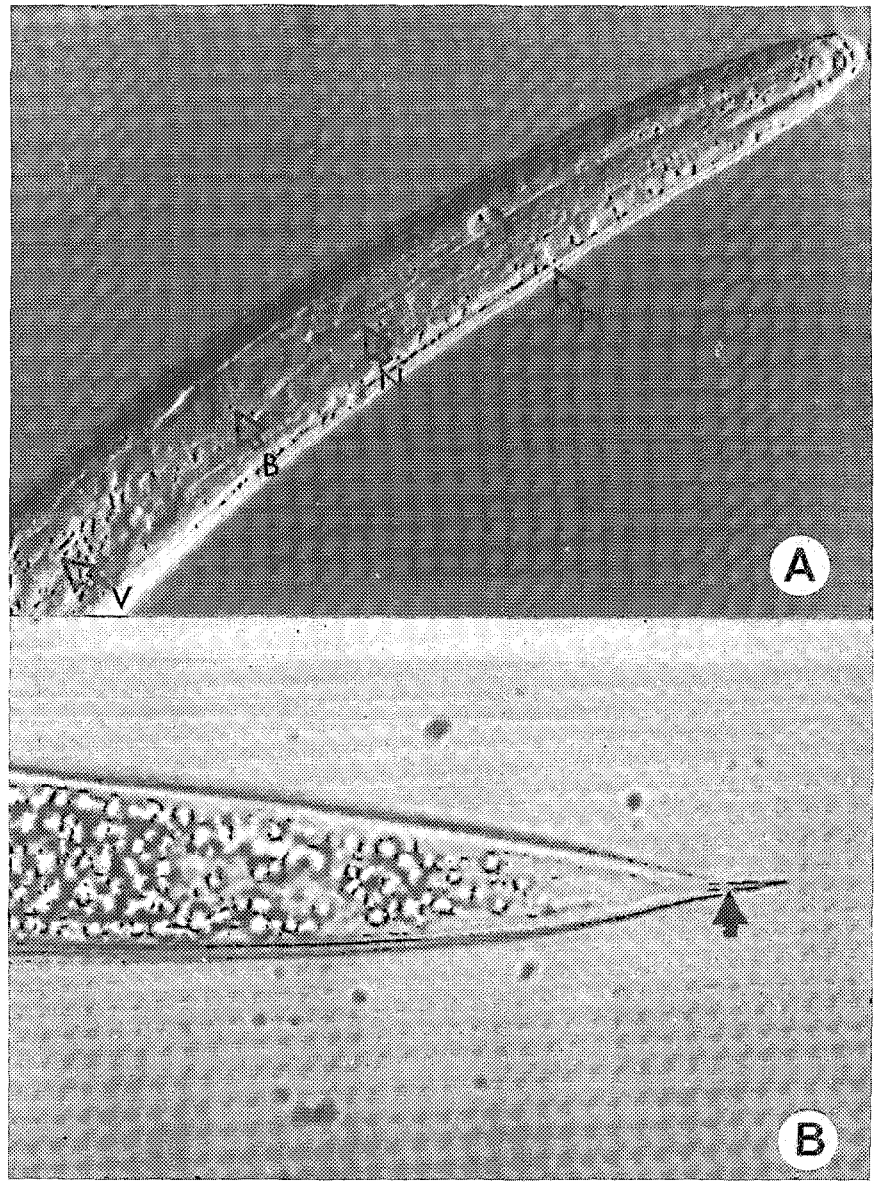

Fig. 2. *Neoaplectana affinis* Bovien, 1937. Infective stage. A : Anterior portion showing excretory pore (P), nerve ring (N), basal bulb of the pharynx (B) and bacterial vesicle (V) (× 770); B : Tip of tail showing terminal spine (arrow) (× 1 200).

females possess a straight tail which often ends in a fine mucron.

Males : With a single reflexed testis. In the seminal vesicle occurred secondary spermatocytes dividing into spermatids and spermatids shedding their residual bodies. The spicules are variable in shape, but tend to be strongly curved and not colored. The tips are narrowed but blunt. The capitulum is broad and generally has a small arch at its base, or the top of the calamus. From this point, the velum extends obliquely nearly to the tip of the lamina. The width and amount of curvature were variable. The gubernaculum was forked with the proximal portions curved upwards and overlapping the spicule laminae. The distal portion varied from simple to hook shaped. The tip of the male tail bears a minute mucron which usually protrudes only as far as the surrounding genital papillae. It is possible to confuse the tail mucron with the genital papillae but the latter are

normally paired. Rarely, an extra-genital papillum occurred on one side and sometimes two central papillae occurred in series. Normally, however, there were the basic complement of twenty-three genital papillae consisting of elevent pairs and a single adanal ventral one. Three pairs occurred near the tail tip surrounding the tail mucron and three pairs occurred in the region posterior to the gubernaculum. Anterior to the cloacal opening, in latero-ventral positions, were a row of five pairs on either side of the body.

Infective stage juveniles : This stage is a third stage juvenile that is often still enclosed in the second stage cuticle. The mouth and anus are closed and intestine are collapsed. Cells of *Xenorhabdus* spp. are contained in a modified vesicle in the anterior portion of the intestinal lumen. All mature infective juveniles bore a minute spine in the tip of their tail.

DIAGNOSIS AND RELATIONSHIPS

Bovien (1937) provided only two quantitative characters of *N. affinis* and these were the length (1.0-1.62 mm) and width (59-100 µm) of the males (no designation regarding whether these were the first or second generation). Additional descriptive remarks were restricted to qualitative characters which separated *N. affinis* from *N. bibionis*. As Bovien (1937) pointed out, *N. affinis* could be separated from *N. bibionis* by : *i)* color of the spicules (grey or colorless in *N. affinis* but orange-brown in *N. bibionis*); *ii)* the slope of the spicules (shorter capitulum and more curved calamus in *N. affinis); iii)* shorter average size of infective stages (693 µm for *N. affinis* and 825 µm for *N. bibionis*) although there is some overlap in ranges and *iv)* the presence of spine in the tip of the tail of the infective stages of *N. affinis* (absent in *N. bibionis).* Bovien (1937) also stated that the gubernaculum of *N. affinis* was more evenly curved without a proximal knob or hook, however in the present investigation, knobbed and hooked gubernacula were observed.

The size of the infective stage, presence of a spine in the infective stage tail, color and shape of the spicules, and presence of a minute cuticular male tail spine separate *N. affinis* from the previously described *N. glaseri* Steiner, *N. carpocapsae* Weiser, *N. intermedia* Poinar, *N. anomali* Kozodoi and *N. rara* Doucet.

The presence of a spine in the tail tip of the infectives is unusual in neoaplectanids. In *N. affinis*, this structure appears only in the fully mature infectives and is very minute. It is now apparent that the form that Poinar (1979) redescribed as *N. bibionis* was in actuality *N. affinis* since the isolations made in Denmark obviously comprised both species and the description fits *N. affinis* as redescribed here. It is interesting to note that the bacterial symbiont of *N. affinis* is very similar to the *Xenorhabdus* symbiont of *N. bibionis.*

TYPE MATERIAL

Bovien (1937) designated no types for *N. affinis.* Through the courtesy of Dr. K. Lindhardt, the author was able to examine Bovien's original slides of *N. affinis* and *N. bibionis.* Bovien designated preserved material of *N. affinis* as " *Neoaplectana* bibio II " and of *N. bibionis* as " *Neoaplectana* bibio I ". Unfortunately the slides were mostly broken and the nematodes had dried, so it was impossible to obtain any meaningful quantitative data from these specimens. However, the present author has designated a lectotype slide containing males of *N. affinis* and two paralectotypes, one with males and one with females. These, as well as topotypes of both sexes from the subsequent collection of Poinar and Lindhardt (1971) are deposited in the Nematology Collection at the University of California, Davis, California, USA.

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