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Recognition of *Neoaplectana* Species (Steinernematidae: Rhabditida)

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ABSTRACT: Morphological characters are presented to separate the males and infective stage juveniles of the four commercially available species of *Neoaplectana* (Steinernematidae: Rhabditidae), namely *N. glaseri, N. bibionis, N. carpocapsae,* and *N. intermedia.* Diagnostic characters in males include the presence and length of the cuticular tail spine and the curvature, nature of the capitulum (manubrium), and tips of the spicules. The infective juveniles can be distinguished by total body length, distance from head to excretory pore, and the ratio distance from head to excretory pore divided by length of tail. A list of the various geographical strains of the above four species is presented.

There are now four well-defined species of *Neoaplectana* that are commercially available and being investigated for use as biological control agents. These are *Neoaplectana glaseri* Steiner, 1929 (redescribed by Poinar, 1978), *N. bibionis* Bovien, 1937 (redescribed by Wouts, 1980), *N. carpocapsae* Weiser, 1955 (redescribed by Poinar, 1967), and *N. intermedia* Poinar, 1985b. The author prefers to utilize the generic name *Neoaplectana* in place of *Steinernema* and *carpocapsae* in place of *feltiae* for reasons presented elsewhere (Poinar, 1984).

The above four species are well established and species determination is based on reproductive isolation, morphological features (Poinar, 1979, 1985b), and DNA analysis (Curran et al., 1985). The previously described species of *Neoaplectana* that are presently not represented by living material will not be considered here because they all require redescriptions.

The need for identification of the above four species has become apparent in commercial operations where more than one species is being produced and accidental mixing can occur. Also in field situations there is often a need to verify the identity of nematodes used. The two stages in the development of *Neoaplectana* that contain diagnostic characters are males and infective juveniles. The present paper describes characters that can be used to separate these stages of the four commercially available species of *Neoaplectana.*

Materials and Methods

The 42 strain of *N. carpocapsae,* the SN strain of *N. bibionis,* the FL strain of *N. glaseri,* and the SC strain of *N. intermedia* were used in this study (Table 1).

The nematodes were grown in larvae of the wax moth (*Galleria mellonella*) maintained at room temperature (~20°C). Males were obtained by dissecting infected insects 5 days after infection. Infective stage juveniles were collected as they emerged from the insect cadavers 10–14 days after infection. All worms were heat killed (50°C), fixed in TAF for 72 hr, and then processed to glycerin. Measurements and photographs were made with a Nikon Orthophot microscope equipped with differential interference contrast optics. For measurements of the infective stage juveniles, 25 individuals of each species were chosen at random from a plate containing several thousand specimens. The ratios used were the standard A (total length divided by width), B (total length divided by distance from head to base of pharynx), and C (total length divided by length of tail). In addition, two other ratios were included; D (distance from head to excretory pore divided by distance from head to base of pharynx) and E (distance from head to excretory pore divided by length of tail).

Results

Three of the four *Neoaplectana* species discussed here are composed of a number of strains (Table 1). These strains interbreed and thus occupy an intraspecific rank. Although it is often impossible to distinguish between strains of the same species on the basis of morphology, it is important to keep them separate because they may differ in host preference, physiology, and behavior. Strains represent populations from a particular host and/or locality. Although strains have been called after the collector (e.g., ALL strain) and the accession number (DD-136), it is preferable to use a name representing the locality or host insect.

The variation found among the adults of a single population from a single insect host prohibits the use of most quantitative characters in separating *Neoaplectana* species. Certain qualitative characters clearly separate the males of the four *Neoaplectana* species being considered. They include the presence and size of the cuticular spine on the end of the male tail and the shape

Table 1. Strains of *Neoaplectana* species.

Species	Strain	Original source	Geographical area	Reference
N. carpocapsae	Czechoslovakian	*Laspeyresia pomonella* (L.)	Czechoslovakia	Weiser, 1955
	DD-136	*L. pomonella* (L.)	Virginia, U.S.A.	Dutky and Hough, 1955
	Mexican	*L. pomonella* (L.)	Allende, Chihuahua, Mexico	Collected by L. Caltagirone
	Sierra	*L. pomonella* (L.)	California, U.S.A.	Collected by A. Berlowitz
	Agriotos (Leningrad)	*Agriotes lineatus* L.	Leningrad, U.S.S.R.	Poinar and Veremtchuk, 1970
	All	*Vitacea polistiformis* (Har.)	Georgia, U.S.A.	Collected by All (in Poinar, 1979)
	XI	*L. pomonella* (L.)	Poland	Stanuszek, 1974
	X-III	*Agrotis segetum* (Schiff)	Poland	Stanuszek, 1974
	X-IV (Pieridarum)	*Pieris brassicae* (L.)	Poland	Stanuszek, 1974 and Akhurst 1983b
	Breton	*Otiorhynchus sulcatus* Fab.	France	Collected by C. Laumond
	Umea	Soil	Sweden	Collected by A. Pye
	42	Cross between Breton and DD-136		Poinar
	Italian	Soil	Italy	Collected by A. Kovec
	Hopland	Soil	California, U.S.A.	Collected by R. S. Lane
	Quebec	*Listronotus oregonensis* (LeConte)	Quebec, Canada	Belair et al., 1984
	N.C.	Soil	North Carolina, U.S.A.	Akhurst and Brooks, 1984
	Nelson	*Vespula* sp.	Tasmania, Australia	Akhurst, 1980
	Powranna	Soil	Tasmania, Australia	Akhurst, 1983a
	Murrumbateman	Soil	New South Wales, Australia	Akhurst, 1983a
	P7	Soil	Tasmania, Australia	Akhurst, 1980
	N55	Soil	Tasmania, Australia	Akhurst, 1980
	Argentinian	*Graphognathus leucoloma* Boh.	Argentina	Ahmad, 1974
	Rhagolites	*Rhagolites pomonella* (L.)	Massachusetts, U.S.A.	Poinar et al., 1977
N. glaseri	N.J.	*Popillia japonica* (L.)	New Jersey, U.S.A.	Glaser, 1932
	N.C.	*Strigoderma arboricola* (Fab.)	North Carolina, U.S.A.	Poinar and Brooks, 1977
	FL.	Soil	Florida, U.S.A.	Collected by O. Sosa
N. bibionis	KL	*Bibio* spp.	Denmark	Bovien, 1937; Poinar and Lindhardt, 1971
	SN	Soil	France	Collected by C. Scotto la Massese
	NZ	*Graphognathus leucoloma* Boh.	New Zealand	Wouts, 1980
	N60	Soil	Canberra, Australia	Molyneux et al., 1983
	T335	*Otiorhynchus sulcatus* Fab.	Tasmania, Australia	Molyneux et al., 1983
	Dutch	Soil	Holland	Galle, 1983
	Murrumbateman	Soil	New South Wales, Australia	Akhurst, 1983a
	Dover	Soil	Tasmania, Australia	Akhurst, 1983a
	T231 (Risdon)	Soil	Tasmania, Australia	Akhurst, 1983b
	Nive strain	Soil	Tasmania, Australia	Akhurst, 1983a
	T298 (Plenty)	Soil	Tasmania, Australia	Akhurst, 1983b
	Bruny strain	Soil	Tasmania, Australia	Akhurst, 1983a
	T319 (Wellington)	Soil	Tasmania, Australia	Akhurst, 1983b
	VI	Soil	Victoria, Australia	Akhurst, 1980
N. intermedia	S.C.	Soil	South Carolina, U.S.A.	Poinar, 1985b

Table 2. Measurements of the infective stages of *N. carpocapsae*.

Character	42 strain ($N = 25$) (present study)	Czechoslovakian strain ($N = 25$) (Poinar, 1967)	Polish strain (Stanuszek, 1974)	DD-136 strain ($N = 25$) (Poinar, 1967)
Total length	544 (502–608)	572 (488–613)	572 (498–650)	547 (438–625)
Greatest width	25 (22–29)	26 (25–28)	23 (20–30)	24 (22–28)
Distance: head to excretory pore	33 (30–37)	42 (39–56)	42 (38–51)	36 (34–40)
Distance: head to nerve ring	80 (76–91)	88 (84–93)	87 (80–99)	85 (81–90)
Distance: head to pharynx base	126 (118–133)	—	115 (103–190)	—
Length tail	55 (51–59)	53 (47–59)	50 (46–61)	53 (50–59)
Anal diameter	13 (11–14)	—	14 (10–16)	—
Ratio A	21 (19–24)	—	—	—
Ratio B	4.4 (4.0–4.8)	—	—	—
Ratio C	10.0 (9.1–11.2)	—	—	—
Ratio D	0.26 (0.23–0.28)	—	—	—
Ratio E	0.60 (0.54–0.66)	—	—	—

and character of the spicules. Characters used to separate males of *Neoaplectana* are presented in the following key and shown in Figures 9–12.

Infective stage juveniles of *Neoaplectana* are less variable in size and shape and quantitative measurements can be used for specific diagnosis. Measurements of the infective stages of one strain of the four studied *Neoaplectana* species, together with previous measurements from the literature, are presented in Tables 2–4. The four species can be recognized by the length of the infective stage juveniles if at least 10 individuals are measured and the average value used. Another important measurement is the distance from the head to the excretory pore. This value is relatively short in *N. carpocapsae* in comparison with the other species and relatively long in *N. glaseri*. Other figures, such as the distance from the head to nerve ring, head to pharynx base, anal diameter, length of tail and greatest width can be used in some instances. The ratios A, B, and C were too variable to be of assistance. However, ratio E showed no overlapping in the strains of four species studied here and is considered of value in separating these and possibly other *Neoaplectana* species. On the basis of characters evaluated in the present study, the following keys to infective stage juveniles and males are presented. Available values reported in the literature for these four species are also incorporated into the present data in the keys.

Key to Infective Stage Juveniles

1. Average length of infective stages ($N = 10$) greater than 725 μm (Figs. 1, 2) 2
   Average length of infective stages ($N = 10$) less than 725 μm (Figs. 3, 4) 3
2. Length generally around 1,200 μm but can vary from 860 to 1,500; distance from head to the excretory pore 85–110 μm; distance from head to base of pharynx 158–168 μm; E ratio from 1.22 to 1.38 ..... *N. glaseri* (Figs. 1, 5)
   Length generally around 800 μm but can vary from 700 to 1,000; distance from head to excretory pore, 53–67 μm; distance from head to base of pharynx 115–150 μm; E ratio from 0.69 to 0.86 ..... *N. bibionis* (Figs. 2, 6)
3. Length generally around 550 μm but can vary from 438 to 650; distance from head to excretory pore 30–56 μm; E

Table 3. Measurements of the infective stages of *N. bibionis*.

Character	SN strain ($N = 25$) (present study)	NZ strain ($N = 20$) (Wouts, 1980)
Total length*	817 (736–896)	880 (750–950)
Greatest width	26 (25–29)	25 (22–27)
Distance: head to excretory pore	61 (56–66)	62 (53–67)
Distance: head to nerve ring	98 (88–112)	100 (89–108)
Distance: head to pharynx base	136 (128–147)	135 (115–150)
Length tail	79 (70–88)	83 (71–92)
Anal diameter	17 (16–19)	16 (15–17)
Ratio A	31 (29–33)	—
Ratio B	6.0 (5.3–6.4)	—
Ratio C	10.4 (9.2–12.6)	—
Ratio D	0.45 (0.42–0.51)	—
Ratio E	0.78 (0.69–0.86)	—

* Bovien (1937) gave a value of 700–1,000 for the KL strain of *N. bibionis*.

Table 4. Measurements of the infective stages of *N. glaseri* and *N. intermedia*.

Character	*N. glaseri*		*N. intermedia*	
	FL. strain ($N = 25$) (present study)	N.C. strain ($N = 10$) (Poinar, 1978)	S.C. strain ($N = 25$) (present study)	S.C. strain ($N = 25$) (Poinar, 1985b)
Total length	1,200 (1,000–1,300)	1,060 (864–1,448)	661 (608–704)	680 (608–800)
Greatest width	40 (31–45)	45 (35–50)	29 (25–32)	28 (25–32)
Distance: head to excretory pore	104 (97–110)	100 (87–108)	64 (59–68)	65 (61–69)
Distance: head to nerve ring	120 (112–126)	—	94 (88–99)	92 (85–96)
Distance: head to pharynx base	162 (158–168)	—	125 (112–133)	121 (110–131)
Length tail	80 (72–86)	76 (62–87)	67 (60–74)	64 (53–72)
Anal diameter	23 (19–24)	—	15 (13–18)	16 (13–18)
Ratio A	29 (26–35)	—	23 (20–26)	—
Ratio B	7.3 (6.3–7.8)	—	5.3 (5.0–6.0)	—
Ratio C	14.7 (13.6–15.7)	—	10.0 (9.3–10.8)	—
Ratio D	0.65 (0.58–0.71)	—	0.51 (0.48–0.58)	—
Ratio E	1.31 (1.22–1.38)	—	0.96 (0.89–1.08)	—

ratio, 0.54–0.66 ..... *N. carpocapsae* (Figs. 4, 8)

Length generally around 650 μm but can vary from 608 to 800 μm; distance from head to excretory pore 59–69 μm; E ratio, 0.89–1.08 ..... *N. intermedia* (Figs. 3, 7)

Key to Males

1. Tip of tail lacking a cuticular spine (Figs. 9, 11) 2
   Tip of tail bearing a cuticular spine (Figs. 10, 12) 3
2. Tip of spicules with a ventral notch, hook or scar; spicules moderately curved (angle between calomus and lamina between 50° and 70°) ..... *N. glaseri* (Fig. 9)
   Tip of spicules bluntly rounded; spicules strongly curved (angle between calomus and lamina between 70° and 90°) ..... *N. intermedia* (Fig. 11)
3. Tail spine 1–4 μm long; spicules grey-yellow; capitulum distinct ..... *N. carpocapsae* (Fig. 12)
   Tail spine 4–13 μm long; spicules yellow-orange; capitulum indistinct ..... *N. bibionis* (Fig. 10)

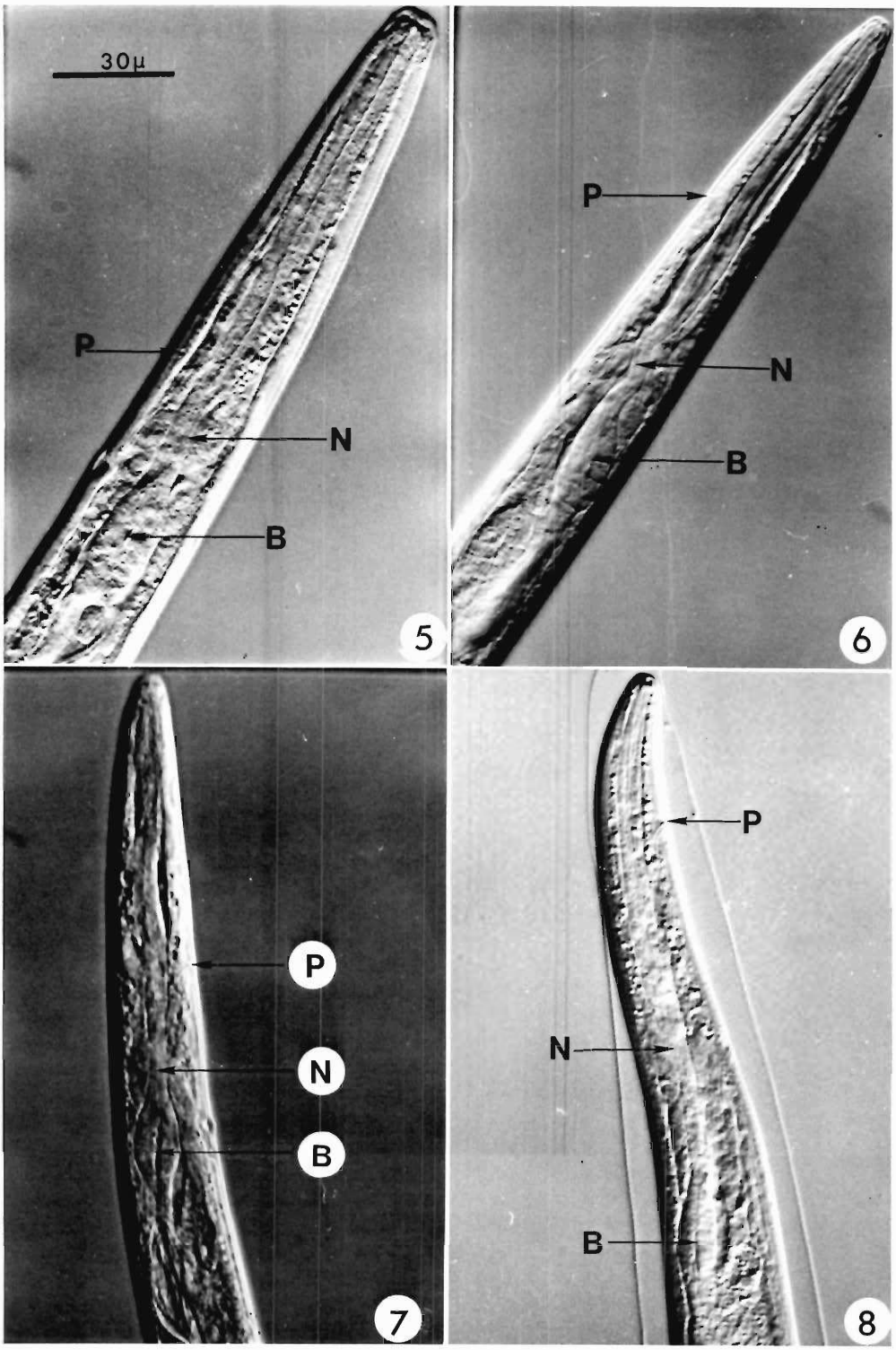
Discussion

With the commercialization and widespread field testing of nematodes belonging to the genus *Neoaplectana* (Poinar, 1985a), there is a growing need for quick methods of identification of the species now in use. The first described species, *N. glaseri,* has been recovered from North America and possibly the Soviet Union (Poinar, 1979). The present distribution of *N. bibionis* includes Europe, Australia, and New Zealand and that of *N. intermedia,* North America. The greatest distribution of the four species is found in *N. carpocapsae,* which has been recovered from North America, Europe, Mexico, Australia, and South America.

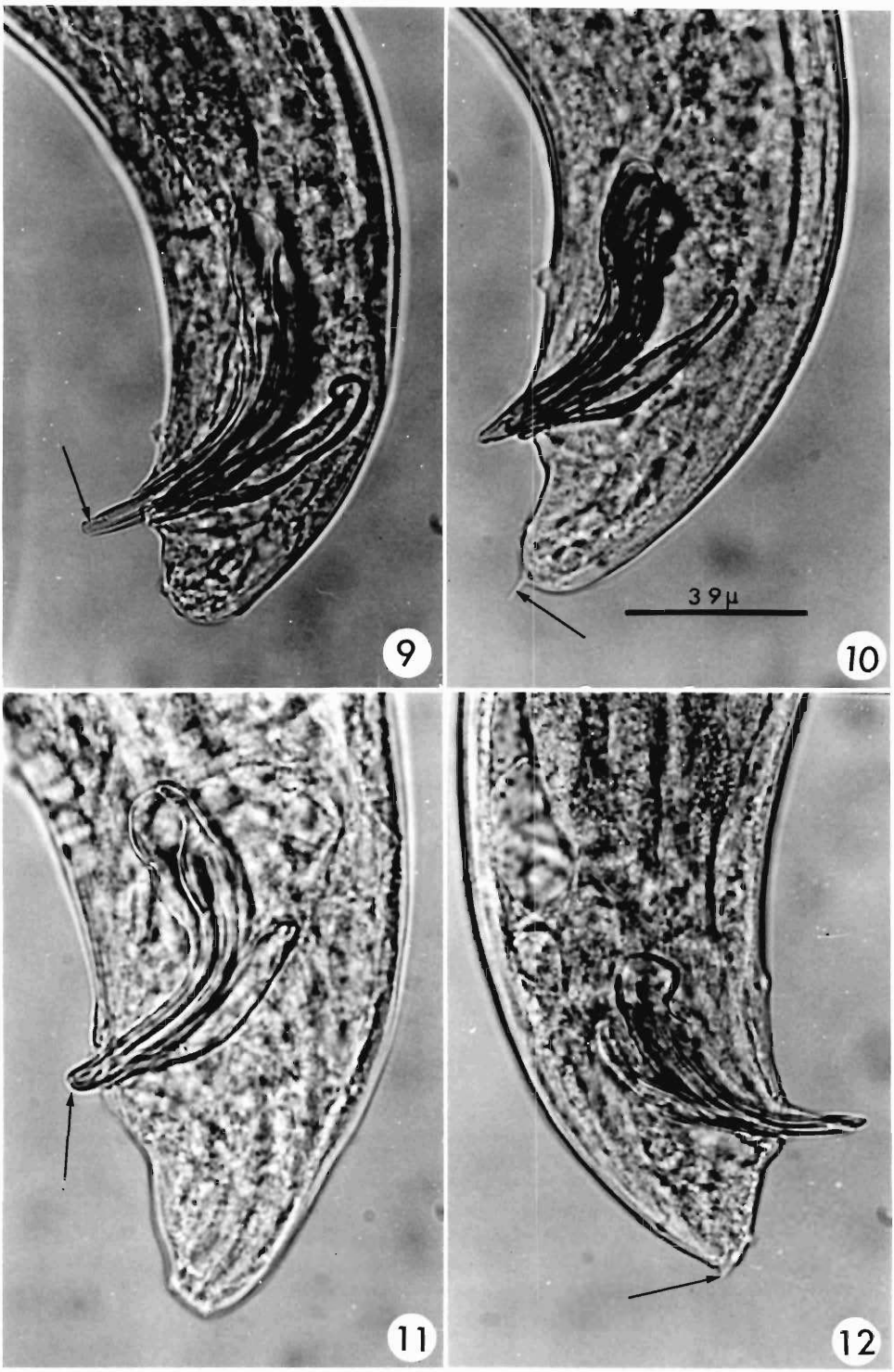
Previous studies (Poinar, 1967, 1978, 1985b; Wouts, 1980) showed that the variability in size found amongst adults of the same *Neoaplectana* species prohibits the use of quantitative characters in distinguishing these stages. Ratio D (distance from the head to the excretory pore divided by the distance from the head to the base of the pharynx) has been used as a specific character for adults (Poinar, 1979) and can be reliable if the sex and generation of the specimen is also known (Poinar, 1985b). It can also be used to separate the infective juveniles of some neoaplectanids. The most reliable ratio, which has shown no overlap in the populations of infective juveniles compared in the present paper, is the distance from the head to the excretory pore divided by the tail length (ratio E).

Although the total length is a fairly reliable character for distinguishing infective juveniles of the four species, there can be some overlap and the range will undoubtedly increase when measurements of the various strains are present. Another possible source of variation arises when the nematodes are cultivated on artificial media. Populations on spent media produce smaller in-

Figures 1–4. Infective stage *Neoaplectana*. *N. glaseri* (1), *N. bibionis* (2), *N. intermedia* (3), and *N. carpocapsae* (4). All photos at same magnification.



Figures 5–8. Pharyngeal regions of infective stage *Neoaplectana. N. glaseri* (5), *N. bibionis* (6), *N. intermedia* (7), and *N. carpocapsae* (8). Note excretory pore (P), nerve ring (N), and basal bulb of pharynx (B). All photos at same magnification.



Figures 9–12. Male tails of *Neoaplectana* species. 9. *N. glaseri* without tail spine, with moderately curved spicules, and scar on spicule tips (arrow). 10. *N. bibionis* with indistinct capitulum and long tail spine (arrow). 11. *N. intermedia* without tail spine, with strongly curved spicules, and blunt spicule tips (arrow). 12. *N. carpocapsae* with minute tail spine (arrow), pointed spicule tips, and well-developed capitulum. All photos at same magnification.

fectives than those on fresh media (pers. obs.). The range of the infectives under these conditions still require investigation.

The infectives of *Neoaplectana* are easily distinguished from those of the related insect parasite, *Heterorhabditis,* by the position of the excretory pore in relation to the nerve ring. The pore is posterior to the nerve ring in *Heterorhabditis* and anterior in *Neoaplectana.* Other characters such as the paired rhabdions in the stoma area, the longitudinal cuticular situations, and the hook on the tip of the head in *Heterorhabditis* are lacking in *Neoaplectana.*

Distinguishing features in male neoaplectanids are restricted to the secondary sex characters although sperm morphology still requires investigation. The presence, absence, and length of a cuticular tail spine is a reliable character although some variation occurs in the length. Variation also occurs in the shape of the spicules and gubernaculum; however, characters such as the presence of a capitulum and arch and the degree of curvature in the spicules are fairly constant. The nature of the spicule tips (blunt, pointed, or notched) is another reliable character. The gubernaculum also shows some variation in lateral view, yet the dorsal or ventral aspect may prove to be a more reliable character.

Female neoaplectanids have no easily recognizable characters to separate them. Some reliability can be given to the location of the excretory pore and shape of the tail but because of their great variability in size, even these characters should be used with caution.

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