Nematol. medit. (1994), 22: 109-113

Istituto di Nematologia Agraria, C.N.R., 70126 Bari, Italy

LIFE STAGES IDENTIFICATION AND EMBRYOLOGY OF THE VIRUS-VECTOR NEMATODE XIPHINEMA INDEX

by
N. Vovlas and A. Larizza

Summary. *Xiphinema index* life stages are illustrated and differentiated on the basis of body length and, functional and replacement odontostyle. The length of the replacement odontostylet on one stage corresponds to the length of functional odontostylet of the previous stage. Odontostyle length increased from 49±4.6 (38-55) µm for the first stage juveniles to 135±2.8 (128-138) µm for the female and male with a growth rate of 61%, 35%, 30% and 13% compared to the previous stage for J_2, J_3, J_4 and adults respectively. *X. index* completes embryogenic development in 10-12 days at 24±2 °C. The embryogenic development starts shortly after egg laying and the 2- and 4-cell stages are reached after 22% of the total embryogenic development time. Thereafter divisions are more rapid, with gastrulation and juvenile after about half (57%) of the total developmental time. The juvenile moves actively during elongation (s-stage phase) which is followed by 2-3 days quiescence. Shortly before hatching, activity is resumed and is accompanied by an evident flexibility of the egg membrane.

In spite of its importance as a natural vector of the grapevine fanleaf nepovirus (GFLV), the life cycle and the embryogenic development of *Xiphinema index* Thorne *et* Allen is not completely known. Moreover, since virus infectivity is lost when vector nematodes moult, a correct identification of the juvenile stages might be necessary during trials of virus transmission. Such identification is made on odontostyle and replacement odontostyle length.

In this paper we identify the life stages of *X. index* in relation to odontostyle formation and the growth rate of the different juvenile stages and adults. The embryogenic patterns were also studied to determine the intervals between egg laying and hatching of the first stage juvenile in a water-agar medium.

Materials and methods

Xiphinema index populations were cultured on fig (*Ficus carica* L.) under glasshouse conditions at 24-26 °C. Eggs, juveniles and adults were extracted from the soil by magnesium sulphate centrifugation (Coolen, 1979). Stage identification was done on nematodes extracted from soil, killed by gentle heat and fixed in 4% formaldehyde + 1% propionic acid, then dehydrated and permanently prepared according to Seinhorst's rapid method (Seinhorst, 1962).

Eggs used in this study were obtained from soil or laid in 2% water-agar directly by gravid females. Embryogenic development was studied in Petri dishes (with 2% water-agar) maintained at room temperature 22-24 °C, examined and observed every 12 hours and microphotographed.

Terminology of the differents parts of the spear (stylet) is according to Coomans and De Coninck (1963), using the term odontostyle for the functional anterior part of the spear, odontophore for the basal portion and replacement odontostyle for that which is present in the oesophagus of juvenile stages and will become functional after moulting. The abbreviations J_1, J_2, J_3, J_4 are used throughout the text and tables to denote the first, second, third and fourth juvenile stages, respectively.

Results and discussion

Morphometric and allometric characters regarding the process of spear development at different stages of *X. index*, directly extracted from the pot culture, are in Table I.

The four juvenile stages were distinguished from each other on the basis of their body length, tail shape and length of functional and replacement odontostyle. The body length of *X. index* increased from 846±71 (760-963) µm for the newly hatched J_1 juveniles to 3152±205 (2716-3533) µm of female. The body length of each motile stage increased by 41%; 58%; 32%; and 26% compared to that of the previous stage after the moults of J_1, J_2, J_3, J_4 respectively (Fig. 1).

Odontostyle length increased from 49±4.6 (38-55) µm

TABLE I - *Main morphometrical characters of juveniles and adults of* Xiphinema index (*all measurements in µm except a, b, c, c' ratios).*

	J1		J2		J3		J4		Female		Male
	x±s.d.	range	x±s.d.	range	x±s.d.	range	x±s.d.	range	x±s.d.	range	
n	11		12		5		6		15		1
L	846.4±71.65	760.0-963.3	1194.4±85.99	1100.0-134.0	1894.0±160.94	1626.7-2036.7	2500.0±261.40	2113.3-2806.7	3152.7±205.44	2713.3±3533.3	3147
Maximum body width	20.7±2.48	17.1-23.7	28.3±4.14	23.7-36.8	38.7±6.05	30.4-43.4	45.6±3.45	40.0-50.0	51.±3.31	46.7-56.7	55.3
Odontostyle	49.3±4.64	38.3-55.0	63.8±2.64	61.7-70.0	87.3±2.80	83.3-90.0	111.6±3.14	108.3-115.0	133.5±2.87	128.3-138.3	133.0
Odontophore	35.3±1?71	33.0-37.0	43.7±5.19	33.3-48.3	57.3±0.88	56.7-58.3	65.8±1.39	65.0-68.3	76.3±3.68	65.0-80.0	71.7
Replacement odontostyle	65.1±3.17	60.4-69.5	90.0±3.68	82.4-94.1	108.1±7.50	101.6-120.6	135.2±7.65	124.2-145.2	–	–	–
Anterior end to guiding ring	33.9±4.85	23.3-40.0	54.7±2.57	50.0-58.3	76.7±2.42	75.0-80.8	97.9±3.56	93.3-103.3	122.9±5.25	112.5-131.7	116.7
Oesophageal length	230.8±8.66	211.7-240.0	296.5±28.30	225.0-325.0	364.0±23.99	331.7-396.7	463.6±24.95	405.0-475.0	489.7±29.39	458.3-556.6	438.3
Tail length	38.0±6.00	26.7-47.5	42.0±4.08	35.0-48.3	52.3±4.67	46.7-56.7	46.9±3.04	41.7-50.0	46.9±7.24	31.7-60.0	45.0
Anal body width	13.1±1.79	10.0-15.0	19.3±2.79	16.6-26.7	29.7±4.62	25.0-35.0	35.09±1.42	33.3-36.7	40.9±3.13	35.0-45.8	41.7
a	41.0±2.46	38.5-45.0	42.6±3.20	35.5-46.4	49.5±4.40	45.8-54.8	54.8±3.42	50.0-60.1	61.5±4.26	54.3-68.0	56.9
b	3.7±0.36	3.2-4.1	4.1±0.70	3.5-5.7	5.2±0.60	4.6-6.1	5.8±0.82	4.8-6.6	6.5±0.58	5.5-7.6	7.2
c	22.4±3.27	18.6-28.7	29.1±4.75	23.5-37.3	36.3±3.60	33.0-42.2	53.4±6.24	43.8-62.4	68.7±10.94	55.9-96.0	69.9
c'	2.9±0.34	2.3-3.4	2.2±0.33	1.5-2.5	1.8±0.30	1.5-2.3	1.3±0.13	1.1-1.5	1.1±0.14	0.8-1.4	1.0

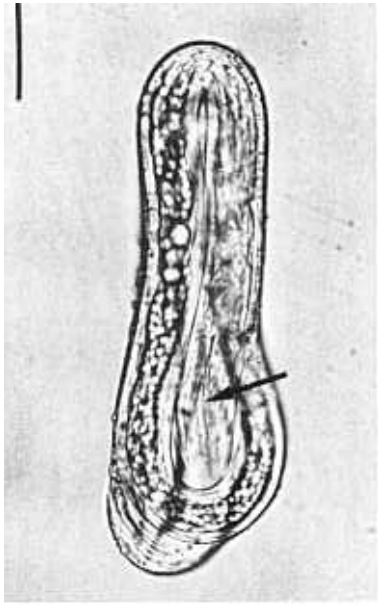

Fig. 2 - A *X. index* egg just before hatching (odontostyle arrowed). Scale bar=30 µm.

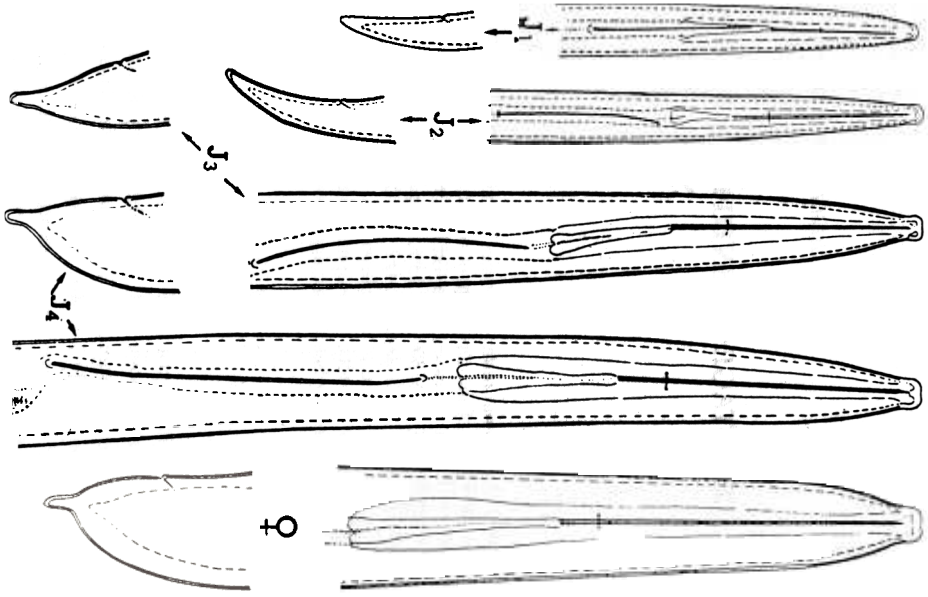

Fig. 1 - Oesophageal region and tail of *Xiphinema index* J_1, J_2, J_3, J_4 juvenile stages and female. Scale bar=60 µm.

for J_1, to 135±3 (128-138) µm for the female and male, with a growth rate of 61%, 35%, 30% and 13% compared to the previous stage for J_1, J_2, J_3, J_4 and adults, respectively

During each juvenile life stage the replacement odontostyle is stored in the slender part of the oesophagus (Fig. 3). The length of the replacement odontostyle is similar to the functional odontostyle in the following life stage (Table I).

Moulting was observed on 45 juveniles placed in water agar. The exsheathment process in *X. index* (Fig. 6) does not differ from that observed in other members of the subfamily Longidorinae (Radewald, 1962; Lamberti, 1969).

Embryology

Eggs obtained from the soil or from gravid females were maintained in 2% water-agar in Petri dishes under a 20 mm diameter coverglass for embryogenic studies. The developmental patterns of *X. index* are given in detail in Fig. 5, and are based on 35 eggs, of which 4 were observed to hatching.

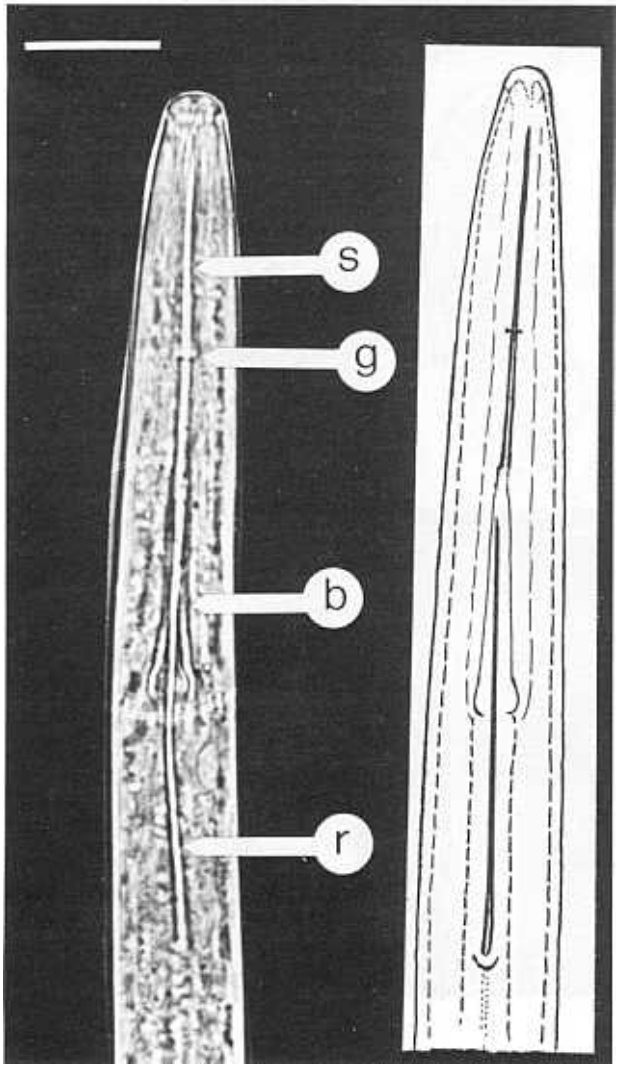

Fig. 3 - Oesophageal region of the first juvenile of *X. index*; s = functional odontostyle, g = guiding ring, b = odontophore, r = replacement odontostyle. Scale bar = 20 µm.

The structure in per cent of egg population of *X. index*, under optimum glasshouse conditions (24-26 °C) with fig as host, in June, revealed that 2% of the eggs were at single cell-stage; 4% at 2-4 cells-stage; 7% multicellular; 25% gastrula; 7% becoming vermiform juveniles; 17% vermiform juveniles and 38% at fully developed first stage juveniles.

Egg measurements

Intra-uterine eggs (n=10): Length = 171±6 (161-179) µm; width = 32±3 (25-34) µm; L/W ratio = 5.4±0.5 (5-6).

Embryonated eggs (n=19): Length = 154±4 (149-161) µm; width = 45±2.3 (40-50) µm; L/W ratio = 3.5±0.2 (3.1-3.8).

Odontostyle of first stage juvenile inside the egg shell = 49±5 (38-55) µm. Fully-formed first stage juveniles folded 3-4 times within the egg shell (Fig. 3).

The single-cell stage (Fig. 4b) can be distinguished by the single nucleus, which is well-visible in many intra-uterine eggs.

Generally, within 24-72 hours after laying, the single cell divides into two cells more or less equal in size (Fig. 4e). Thereafter divisions are very rapid and the 2-4 cells stage is reached after 22% of the total time of development. The phases of the subsequent divisions does not seem to be uniform and no defined pattern of gastrulation was observed. The anterior region of the gastrula stage is pale with undefined cells, while the posterior region is darker with small cells with fine and granular content. Development from single-cell stage to gastrula (Fig. 4f) occupied 4-5 days.

The folded vermiform juvenile occupies the length of the egg and 48 hours later elongates rapidly, moving actively and when it has attained approximately two or three folds the oesophagus begins to differentiate and the odontostyle tip becomes visible in the anterior body portion. Growth from the twice-folded stage of the juvenile to fully formed J_1 (4 folds for *X. index*) is rapid, taking $2^1/_2$ days.

In fully-formed juveniles the guiding ring of the primary odontostyle becomes visible (Fig. 3) and the flanges of the posterior portion of the odontophore begin to form. In the first juvenile stage inside the egg the replacement odontostyle is fully-formed 12 days after the first cell division.

For 24-36 hours after its formation, the juvenile continues to move actively within the egg, and the egg-shell appears to be flexible; the egg diameter increases slightly, and a swelling on one side of the egg membrane shortly before membranes rupture is observed (Fig. 2).

The developmental pattern of our population of *X. index* cultured on fig is similar to those described for some *Xiphinema* and *Longidorus* species by Flegg (1968).

The embryogenic period observed (12 days at 24 °C) is slightly longer than that reported for *X. index* (about a week)

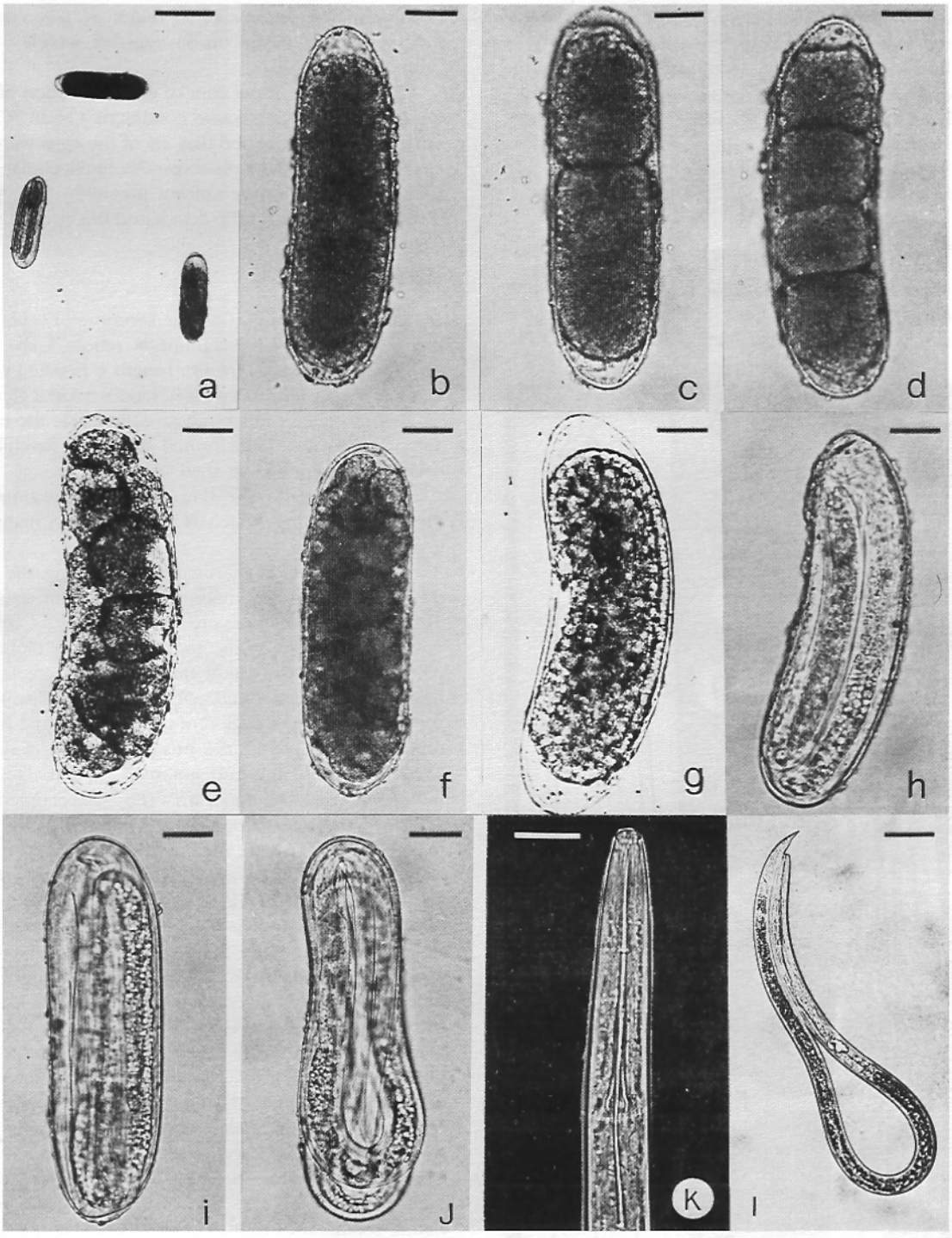

Fig. 4 - Embryogeny of *X. index*: a, developmental stages of eggs on water-agar medium; b, single-cell phase; c, 2-cells phase just before the second division; d, 4-cells phase; e, multicellular stage; f, gastrula; g, juvenile becoming vermiform; h, early stage of vermiform juvenile; h and i, juvenile with developing replacement odontostyle; j, juvenile just before hatching; k and l, anterior end and entire first stage (J_1) juvenile after hatching in agar. Scale bars: a = 100 µm; b-k = 20 µm; l = 50 µm.

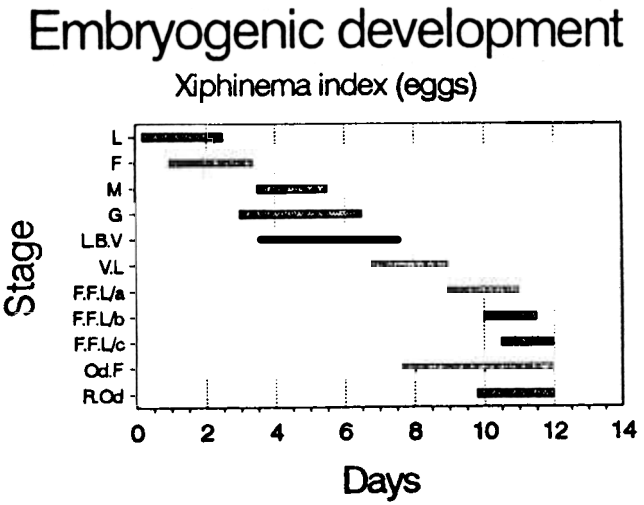

Fig. 5 - Time of the development of *X. index* eggs placed in 2% water agar Petri dishes at 22-24 °C; L = laying; F = 2-4 cells; M = multicellular; G = gastrula; L.B.V. = juvenile becoming vermiform; V.L. = vermiform juvenile; F.F.L./ = fully formed juvenile (a = active, b = passive, c = active); Od. F = odontostyle formation; R.Od = replacement odontostyle formation.

by Radewald (1962) and Fisher (1962). The highly active period (2-3 days) of the fully formed juvenile before hatching in agar plates is about 35% of the total embryogenic developmental time and is not comparable with Radewald's and Fisher's observations which refer to soil environments.

Literature cited

COOLEN W. A., 1979. Methods for the extraction of *Meloidogyne* spp. and other nematodes from roots and soil. *In*: Root-knot Nematodes (*Meloidogyne* species), Systematics, Biology and Control. Eds F. Lamberti and C. E. Taylor. Academic Press, London, pp. 317-329.

COOMANS A. and DE CONINCK L., 1963. Observations on spear formation in *Xiphinema*. *Nematologica*, *9*: 85-96.

FISHER J. M., 1962. Biology of *Xiphinema index*. Unpublished report, University of California, Davis.

FLEGG J. J. M., 1968. Embryogenic studies of some *Xiphinema* and *Longidorus* species. *Nematologica*, *14*: 137-145.

LAMBERTI F., 1969. Exsheatment in *Longidorus africanus* (Nematoda: Dorylamoidea). *J. Nematol.*, *1*: 94-95.

RADEWALD J. D., 1962. The biology of *Xiphinema index* and the pathological effect of the species on grape. Ph. D. Thesis, University of California.

SEINHORST J. W., 1962. On the killing, fixation and transferring to glycerine of the namtodes. *Nematologica*: *8*: 23-32.

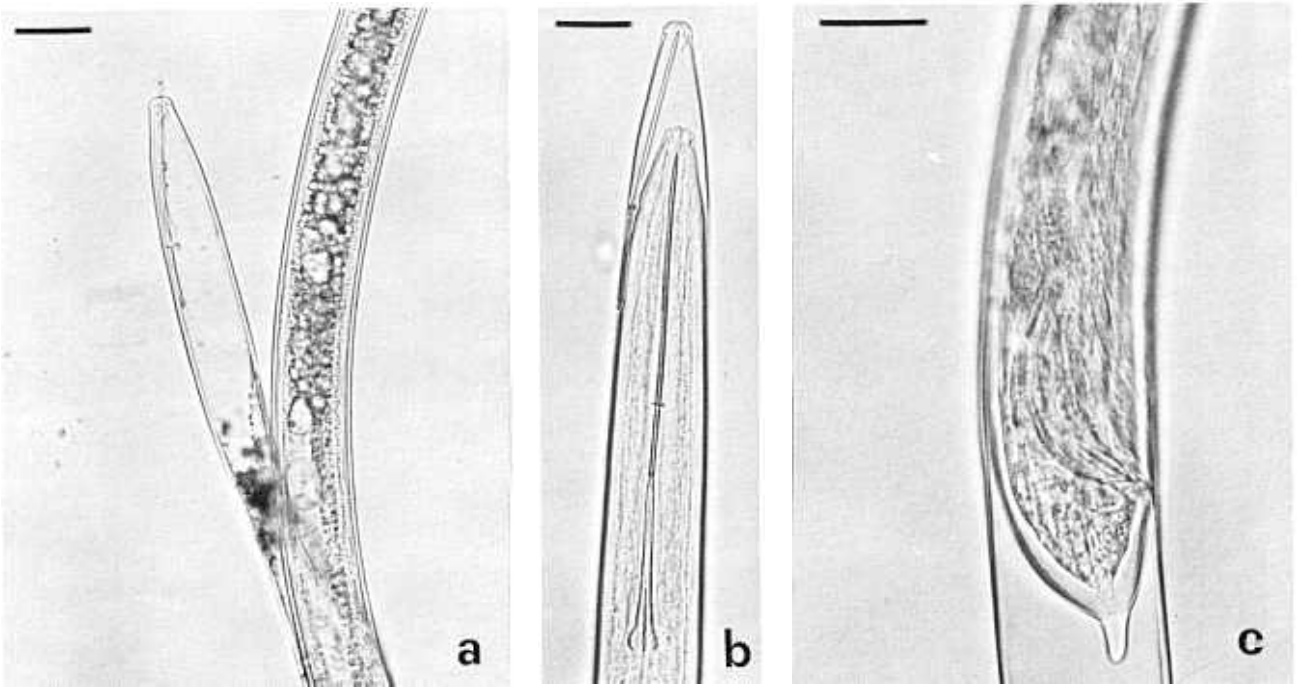

Fig. 6 - Exsheathment stages of *X. index* in juvenile and adult specimens: a, juvenile (J4) specimen emerging from the (J3) cuticle; b, female anterior body portion into the J4 cuticle; c, male posterior body portion into the (J4) cuticle just after fourth moult. Scale bar = 30 µm.

Accepted for publication on 25 March 1994.