

Description of developmental stages of *Hemicriconemoides mangiferae* Siddiqi, 1961 (Nemata : Criconematidae)⁽¹⁾

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SUMMARY

The genital tract length, stylet length and body length were found to be major criteria in differentiating the life cycle stages of *Hemicriconemoides mangiferae*. All juvenile stages except second stage possessed twelve longitudinal rows of spines arranged through the length of the body. The second-stage juvenile possessed a spine-less cuticle. Greatest increase in body length and ovary length was observed between second and third stage and third and fourth stage respectively. Length of stylet increased greatly between fourth and adult stage. Sex differentiation occurred in the fourth stage. Third stage juvenile was identified from second stage by the greater ovary length. A few adult females were observed with single flexure in the distal end of the ovary.

RÉSUMÉ

Description des stades juvéniles de Hemicriconemoides mangiferae Siddiqi, 1961 (Nemata : Criconematidae)

Les principaux critères pouvant servir à différencier les stades juvéniles de *Hemicriconemoides mangiferae* sont la longueur du tractus génital, celle du stylet et celle du corps. Tous les stades juvéniles sauf le deuxième présentent douze rangées longitudinales d'épines sur toute la longueur du corps. La cuticule des J2 est inermes. La plus importante croissance de la longueur du corps et de celle de l'ovaire est observée entre les J2 et les J3, et les J3 et les J4, respectivement. La longueur du stylet s'accroît fortement au passage de J4 à adulte. La différenciation sexuelle a lieu chez les J4. Les J3 peuvent être différenciés des J2 par la plus grande longueur de l'ovaire. Quelques femelles adultes présentent un unique repli de l'extrémité distale de l'ovaire.

Attempts to study the ontogeny and to describe the life-cycle stages of the species of Criconematidae are few and far between (Knobloch, 1978). Due to the inadequate details on the developmental morphology and the existing close relationship between genera of Criconematidae workers have placed juvenile stages across genera (Mehta & Raski, 1971), occasionally describing them as « new species ». Dasgupta, Raski and Van Gundy (1969) emphasised the need to describe the larval stages of all species to understand the relationship between species. The males of most species of *Hemicriconemoides* are uncommon which reiterates the need to describe the juvenile stages at length. The present study describes the individual life cycle stages of *H. mangiferae* Siddiqi, 1961, a parasite of wide range of

crops and most frequently found in tropical and sub-tropical regions.

Materials and methods

Nematodes were extracted by centrifugal-flotation technique (Jenkins, 1964) from soil samples collected from a sapota orchard in Tiruchirapalli, India. Individuals were isolated for study from a single sample containing all stages of *H. mangiferae*. Specimens were processed through Seinhorst solutions (Seinhorst, 1959). Measurements were made using a filar micrometer and from camera lucida tracings. Individual stages were separated by virtue of their body length and gonad length, though a few transitional specimens were confirmed through measurements. All the stages were available for study.

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Results and discussion

MORPHOMETRICS

The body length of *H. mangiferae* increased from 0.1450–0.1733 mm for second-stage juveniles to 0.4094–0.4875 mm for adult females. The growth in body length was greatest between the second and third stages (74.8 %) and least between the fourth and adult stages (24.2 %). The number of body annules was almost constant from stage to stage ($R = 123$, $CV = 4.2$). Annule width increased from 1.9 μm for second stage juveniles to 4.0 μm for adult females. Apparently, the increased annule width resulted in the increased body length from stage to stage. Body width increased from 14.0 μm for second stage to 24 μm for adult females.

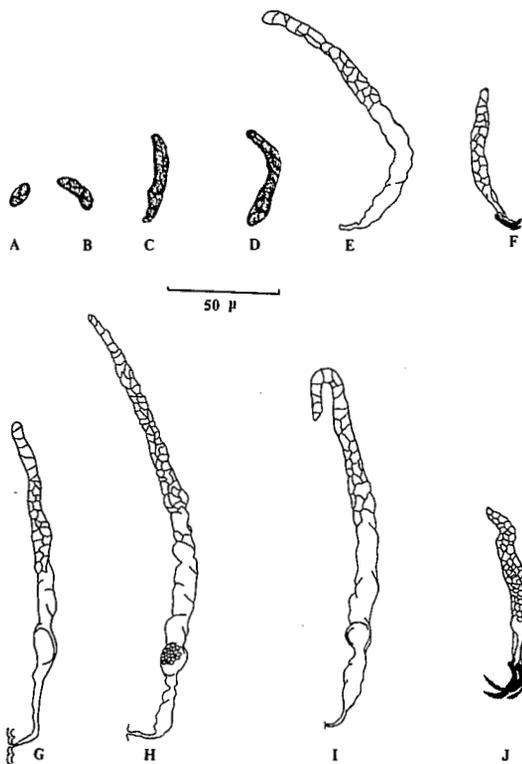


Fig. 1. Development of gonad in *Hemicriconemoides mangiferae*. A : Second stage; B : Late second stage; C : Third stage; D : Late third stage; E : Fourth stage ovary; F : Fourth stage testis; G-I : Adult ovary; I : Reflexed ovary in adult; J : Adult testis.

Growth in body width was greatest between the second and third stage (31.3 %) and least between the fourth and adult stage (8.1 %). The length of stylet increased from 42.7 μm in the second stage to 61.5 μm in the adult female. Growth in stylet length was greatest (16.5 %) between fourth and adult stage and least (10.8 %)

between second stage and third stage. The developmental pattern of the anterior cone portion of the stylet was the same as for the entire stylet. The oesophagus also showed a similar pattern of growth, with increase in length being greatest (22.5 %) between fourth and adult stages and least (8.8 %) between the second and third stages.

The female reproductive system increased from 18.0–23.3 μm for second stage to 56.3–296.9 μm for adult females (Fig. 1). The growth of ovary was greatest between the third and fourth stage juveniles (241.8 %) and least between the fourth and adult stages (38 %).

ALLOMETRICS

The “a” ratio indicated the adult females to be the thinnest and the second-stage juveniles the stoutest. The third and fourth stage juveniles were similar in stature. The “b” ratio increased with nematode maturity being greatest (36.6 %) between second and third stage juveniles, which coincided with the time of greatest increase in body length. Anal openings were obscure in early stages of development, so the “c” ratio was not compared.

DESCRIPTION OF DEVELOPMENTAL STAGES

Second stage juvenile

Body arcuate when fixed with a mean of 119 annules. Annules do not bear spines or scales and are smooth. The head structure was similar to that of adult female with variation only in the width and height of head. The stylet was strongly developed with forward projecting spear knobs and averaged 42.7 μm in total length (Table 1). The genital primodium in the early second stage was almost oval, about 18 μm long. In late second stage, this lengthened in a developing gonad to 23.3 μm . The genital primodium was comprised of four clearly differentiated cells in the early second stage and lengthened to form a six to eight celled sausage-shaped gonad, in the late second-stage. The anus was not seen. The tail was similar in shape to that of the adult tail and varied only in width at the posterior region (12.9 μm).

In moulting, the second stage juvenile cuticle length was 173.3 μm ; the gonad in developing third stage juvenile in second stage (Fig. 1) cuticle was 23.3 μm . Stylet length was 46 μm and anterior cone of stylet 31 μm . A specimen was observed with developing third stage juvenile in second stage cuticle, which possessed spined annules in the posterior region and characters of second stage (smooth cuticle) in the anterior region, indicating that moulting proceeded from the posterior region.

Among the characters, greatest variation was observed in the knob height ($CV = 11.4$) and total body length ($CV = 10.3$).

Table 1
Morphometric and allometric characteristics
of the life-cycle stages of *Hemicriciconemoides mangiferae*

Character	Life Cycle Stage			
	Second (n = 8)	Third (n = 12)	Fourth (n = 14)	Adult female (n = 14)
MORPHOMETRICS (µm)				
Body length	155 (16.0)* (145.0-173.0)**	271 (18.1) (250.0-281.0)	354 (17.9) (344.0-375.0)	439 (3.8) (410.0-488.0)
Body width	14.6 (0.8) (14.2-15.6)	18.8 (2.2) (16.1-20.8)	24.0 (1.4) (22.0-24.0)	26.0 (3.0) (22.0-34.0)
Oesophageal length	66.5 (4.8) (63.7-72.0)	72.3 (5.1) (70.0-78.1)	87.0 (9.3) (78.8-97.0)	106.5 (8.0) (96.8-125.0)
Stylet length	42.7 (2.9) (41.0-46.0)	47.3 (1.5) (46.0-49.0)	53.0 (1.4) (52.0-54.4)	61.5 (5.0) (53.0-74.1)
Prorhabdion length	29.7 (1.2) (29.0-31.0)	40.0 (4.5) (36.0-44.3)	44.3 (3.8) (40.0-47.0)	46.0 (4.7) (41.0-53.6)
Knob height	2.7 (0.3) (2.4-3.0)	3.9 (0.1) (3.8-4.0)	4.2 (0.3) (3.8-4.4)	5.1 (1.8) (4.0-5.0)
Knob width	4.5 (0.4) (4.1-4.8)	4.8 (0.2) (4.7-5.0)	6.9 (1.0) (6.2-8.0)	7.1 (1.3) (6.2-9.0)
Annule width	1.92 (0.1) (1.88-2.0)	2.60 (0.1) (2.50-2.7)	3.60 (0.6) (2.80-4.00)	4.00 (0.3) (3.10-4.40)
Female gonad length	20.0 (3.0) (18.0-23.0)	42.0 (2.7) (40.0-45.0)	143.3 (18.0) (131.3-164.2)	198.0 (41.0) (156.3-296.9)
R	119 (1.2) (118-120)	120 (1.5) (119-122)	125 (2.3) (122-126)	128 (8.0) (125-143)
R ex	27 (0.6) (27-28)	30 (0.7) (26-35)	30 (0.6) (30-31)	32 (3.3) (29-38)
R oes	33 (0.6) (30-33)	34 (1.0) (31-47)	35 (3.5) (33-38)	37 (4.1) (36-43)
RZ	—	—	—	84 (7.4) (70-92)
ALLOMETRICS				
a ratio	10.56 (0.5) (10.2-11.1)	14.43 (0.9) (13.5-15.2)	15.1 (1.0) (14.0-15.6)	17.0 (2.0) (13.9-20.8)
b ratio	2.3 (0.1) (2.3-2.4)	3.8 (0.3) (3.4-4.1)	4.1 (0.7) (3.4-4.8)	4.1 (0.4) (3.7-4.7)
c ratio	—	—	—	16.3 (2.2) (14.2-22.2)

* Mean and standard deviation.

** Range.

Third stage juvenile

Body arcuate to straight when heat relaxed with a mean of 120 annules and twelve rows of spines in each annule arranged longitudinally over the length of the body. Head annules and shape and stylet structure were

similar to those in second stage. In the early stage of development, the third stage genital primordium was 40 μm long with seventeen clearly differentiated cells. No change in tail shape was observed but for the possession of spines on the annules (Fig. 2).

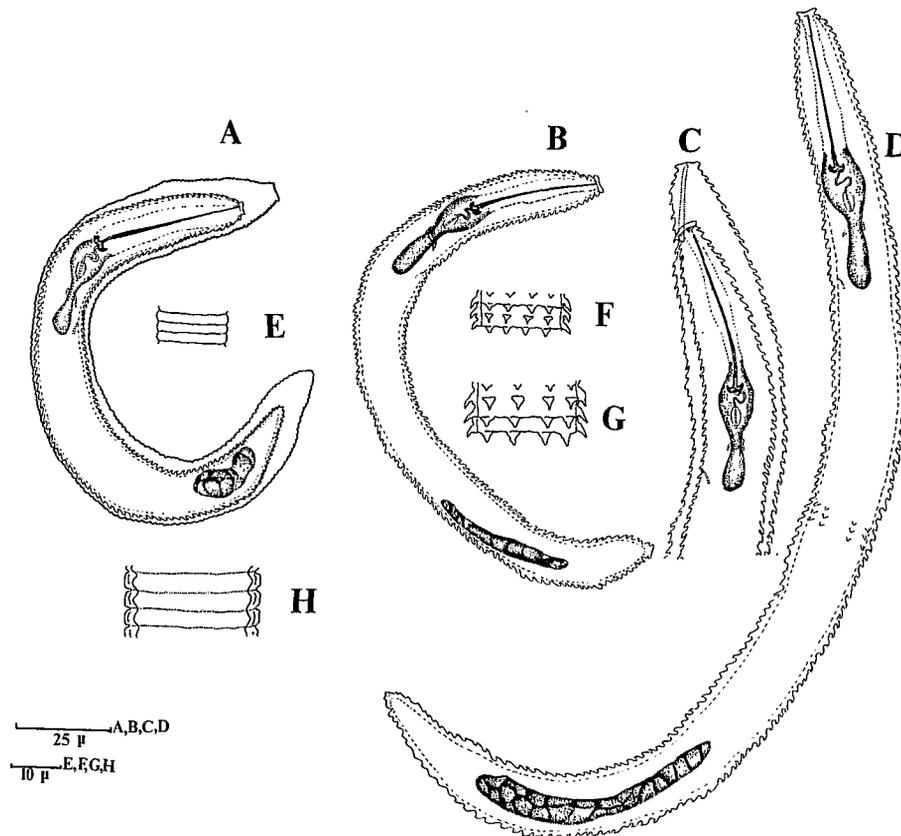


Fig. 2. Juvenile stages of *Hemicriconemoides mangiferae*. A : Developing third stage inside second stage cuticle; B : Third stage; C : Developing fourth stage inside third stage cuticle; D : Fourth stage - E-G : Juvenile cuticle annules. E : Second stage; F : Third stage; G : Fourth stage; H : Adult female cuticle.

The growth in body length was uniform (CV = 6.7) as in second stage. Similar was lengthening on the stylet (CV = 3.2). However, the head height and width showed great variations of 27.7 and 20.4 % respectively, quite unlike the second stage.

Fourth stage female juvenile

Body assumed a variety of shapes, from near straight to slightly arcuate, with a mean of 125 body annules. The annules possessed twelve rows of spines as in the third stage. The shape of head and stylet remained unchanged though their sizes changed with maturity.

The length of stylet ranged from 52-54.4 μm . In this stage the gonad was 143.3 μm long and more developed than in the earlier stages. Genital primordium increased from 25 in early fourth stage to numerous cells in later stage where the region proximal to vagina was hyaline. The germinal zone was clearly differentiated. In some specimens a suggestion of vagina was observed even though vulval opening was wanting.

The pattern of growth of oesophagus was quite dissimilar to that of any other stage, for there was appreciable lengthening of the oesophagus. Due to the above variation, the "b" ratio also showed greater instability (CV = 16.2).

Pre-adult male

Some fourth moults contained males. The fourth stage cuticle was 375 μm with clearly defined testis. The cuticle of pre-adult males also possessed twelve longitudinal rows of scales as in other juvenile stages. Spicule and gubernaculum were well formed measuring 17.2-18.3 μm and 2.8-3.1 μm respectively. Stylet was relatively shorter than that of the fourth stage preadult female juvenile and ranged between 48.0-51.0 μm with a mean of 49.2 μm . However, the stylet was shed along with the cuticle during moulting into adult male, with no new process replacing it.

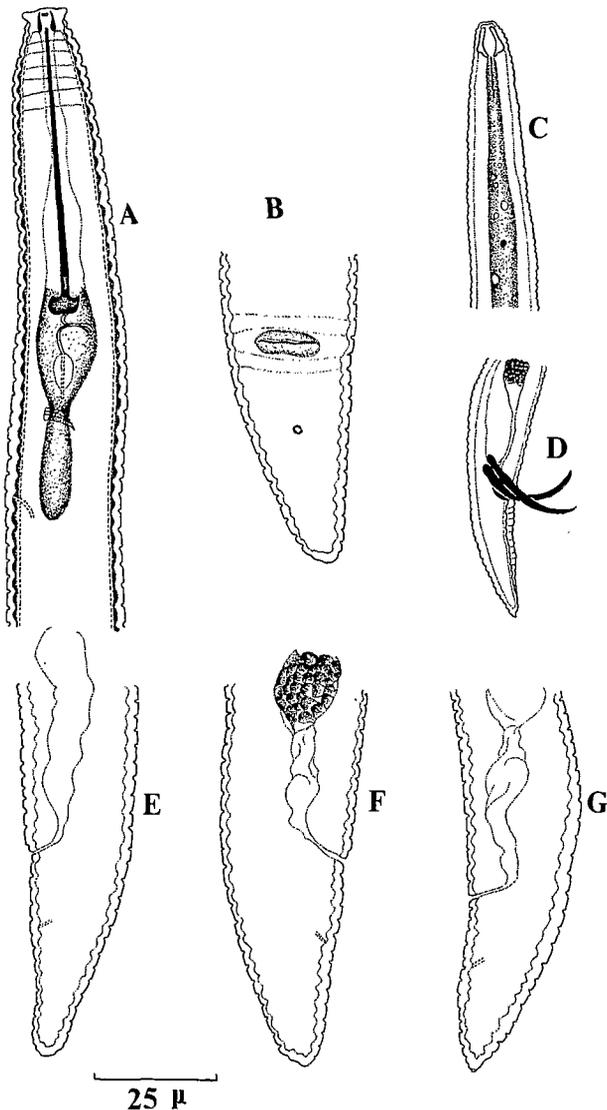


Fig. 3. Adult stages of *Hemicriconemoides mangiferae*. A-B : Adult female; C-D : Adult male; E-G : Tail shapes in adult female.

Sex differentiation occurred at the fourth-stage when well defined testis and ovary were observed. Anal opening was obscure in all juvenile stages.

Adult female

A minor deviation from the original description by Siddiqi (1961) of adult female in *H. mangiferae* was seen in the location of the excretory pore which was five annules anterior to R oes. Similar discrepancy in the location of excretory pore was observed earlier in *Criconemoides parvulus* (Edward & Misra, 1965). Siddiqi (1961, 1977) described two tail shapes (Fig. 3 E & F) in adult females. An intermediate shape (Fig. 3 G) was observed in the same population. Similar tail shapes are not uncommon in other species of *Hemicriconemoides*, which cautions the use of tail shape alone in species identification. Adult females of *Hemicriconemoides* possess outstretched ovary without flexures as a general rule. A few females in the present study showed a single flexure in the distal end which was also observed by Dasgupta, Raski and Van Gundy (1969), in other species.

Adult male

Though males of *H. mangiferae* are documented to be common, only a single adult male was found in the population. Parthenogenic reproduction may be the rule in such cases. The measurements of the individual are L = 358.2 μm ; a = 24.0; b = 5.7; c = 12.0; body width = 14.9 μm ; oesophageal length = 63.4 μm ; tail = 30.0 μm ; testis = 73.3 μm ; spicules = 23.3 μm ; gubernaculum = 4.2 μm ; T = 36.3.

Annules were fine and about 1.5 μm wide near middle of the body. Lip comprised of five annules and a labial disc. Annules of head were continuous with body annules. Stylet absent and oesophagus degenerate. Caudal alae reduced with coarse crenate margins ending before the tail tip.

Fassuliotis (1962) described the life history of *H. chitwoodi* Esser, 1960, to contain three moultings; one in egg, and two in soil. However Dasgupta, Raski and Van Gundy (1969) found four moultings and opined Fassuliotis' observation to have omitted pre-adult stages. Kumar (1980) found sex differentiation to occur in the third stage of *H. cocophilus* (Loos, 1949) Chitwood & Birchfield, 1957, while in *H. mangiferae* and *H. chitwoodi* sex differentiation occurred in the fourth stage. Fassuliotis also observed the spines of juvenile stages of *H. chitwoodi* to possess a 1 μm long setae-like process which is lacking in *H. mangiferae*. The number of rows of spines are the same in juveniles of *H. mangiferae*, *H. chitwoodi* and *H. strictathecatus* Esser, 1960. But information on the shape of spine, gonad and stylet length will be useful in differentiating the former two while the latter could be identified by rounded spear knobs.

Juvenile stages of *Hemicyclophora* do not possess spines nor scales (Brzeski & Zuckerman, 1965). The

possession of spines in the juvenile stages of *Hemicriconemoides* would be useful in separating these two genera. From these observations it may be suggested that *Criconema* and *Hemicriconemoides* are more closely related to each other than to *Hemicycliophora*.

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