

Ontogeny and Identification of Life-Cycle Stages of *Criconema octangulare* (Cobb, 1914) Taylor, 1936

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Abstract: The ontogeny of *Criconema octangulare* (Cobb, 1914) Taylor, 1936, is examined and shows ovary development and body length to be of primary importance in differentiating its life-cycle stages. Second-stage juveniles can be identified by body length and ovary length. The third stage can be distinguished from the second by longer body and ovary length, and from the fourth by a shorter ovary. The fourth stage can be identified by stylet length and ovary length. Included is a key to the life stages of *C. octangulare*, a description of the life-cycle stages, and light and scanning-electron microscopy illustrations. **Key Words:** scanning electron micrographs.

INTRODUCTION

Little is known about the life cycle of most species of the Criconematinae (2), including *Criconema octangulare* (Cobb, 1914) Taylor, 1936. That species was found frequently throughout wooded areas of the Michigan State University Water Quality Project site (MSUWQP), and its ecology is discussed in relation to other taxa in the Criconematinae (5). Soil from MSUWQP contained *C. octangulare* undergoing a series of molts, and all life-cycle stages were available for study. This report describes the life-cycle stages of *C. octangulare* in relation to its ontogeny.

MATERIALS AND METHODS

Soil samples from MSUWQP were collected on August 14, 1976, and processed for nematodes by a modified centrifugation-flotation technique (4). The nematodes were heat-relaxed, fixed in 4% formalin, and transferred to glycerin (7). They were mounted in anhydrous glycerin on slides, and measurements were made with a Wild ocular micrometer and a Wild drawing tube from camera lucida tracings. Nematodes for scanning-electron microscopy (SEM) were critical-point-dried or glycerin-infiltrated, and placed on SEM stubs, and the excess glycerin removed with a Nucleopore filter. The specimens were then coated with gold in a sputter coater (Film-Vac, Englewood, N.J.) and examined in an SEM at an accelerating voltage of 15 KV.

Specimens were collected from a plot which contained only the stylet-bearing nematodes *Xiphinema americanum*, *Lobocriconema thornei*, and *Criconema octangulare*. The juveniles of *C. octangulare* were collected, preserved in glycerin, and measured on slides. These included second-stage ($n = 13$), third-stage ($n = 12$), fourth-stage ($n = 14$), and adult females ($n = 12$). Their measurements fell into separate categories, from which were developed the conclusions as to stages.

RESULTS AND DISCUSSION

ONTOGENY

Morphometrics: The body length of *C. octangulare* increased from 167-214 μm for second-stage juveniles to 331-442 μm for adult females. The growth in body length (48%) was greatest between the fourth and adult stages, and least (14%) between the third and fourth stages. The number of body annules was constant from stage to stage (73, $\sigma = 2.6$) and the growth in body length resulted from increased annule width (Table 1). Body width increased from 22 μm for second-stage juveniles to 43 μm for adult females. Growth in body width (43%) was greatest between the fourth and adult stages, and least (4%) between the second and third stages.

The stylet increased from 31-38 μm for second-stage juveniles to 54-60 μm for adult females. Growth in stylet length (30%) was greatest between the fourth and adult stages, and least (6%) between the second and third stages. The developmental pattern of the anterior or cone portion of the stylet was the same as for the entire stylet. The esophagus showed a different pattern of growth, with the increase in length (19%) being greatest between the second

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TABLE 1. Morphometric and allometric characteristics of the life-cycle stages of *Criconema octangulare*.

Character	Life-cycle stage			
	Second (σ) ^a n ^b = 13	Third (σ) n = 12	Fourth (σ) n = 14	Female (σ) n = 12
<i>Morphometric</i> (μm)				
Body length	188(16)	234(10)	266(20)	393(39)
Body width	22(3)	23(3)	30(4)	43(6)
Annule width	2.5(0.2)	3.2(0.2)	3.6(0.3)	5.6(0.5)
Stylet length	34(2)	36(2)	44(2)	57(2)
Length of stylet cone	25(1)	27(2)	35(2)	43(2)
Esophagus length	69(6)	82(5)	87(4)	101(9)
Female gonad length	16(6)	37(5)	85(13)	262(51)
<i>Allometric</i>				
a ratio ^c	8.5(0.7)	10.0(0.8)	9.0(1.0)	9.0(0.2)
b ratio ^d	2.7(0.2)	2.9(0.2)	3.0(0.3)	4.0(0.3)

^a σ = standard deviation.

^bn = number measured.

^cbody length/body width.

^dbody length/esophagus length.

and third stages, and least (6%) between the third and fourth stages.

The female reproductive system increased from 9.27 μm for second-stage juveniles to 180-342 μm for adult females. Growth of the female gonad was similar between the second and third stages, and third and fourth stages (respectively 134 and 130%), but increased by 208% between the fourth stage and the adult female.

Allometrics: the *a* ratio indicates that the third stage is thinnest, and the second stage the stoutest, while the fourth stages and females are similar in stature (Table 1). The *b* ratio increased with nematode maturity, being greatest (33%) between the fourth and adult stages, the time of greatest increase in body length. The *c* ratio could not be calculated, since anal openings were observed only in mature females.

Metamorphosis: Juveniles of the second, third, and fourth stages had 12-13 scale-like cuticular lobes on each annule in the mid-body region (Fig. 2-I,J). The SEM picture of a juvenile (unidentified stage) indicates that there may be as many as 16 scale-like cuticular lobes (Fig. 1-F). Cuticular metamorphosis occurred during fourth molt, reducing the number of scale-like cuticular lobes to 8 per midbody annule (Fig. 1-B,C,E, 2-K). Two of the scale-like cuticular lobes are located on each of the subdorsal and subventral quadrants.

Identification: The stages in the life cycle of the female can be identified through the number of scale-like cuticular lobes per body annule, body length, stylet length, and ovary length (Table 1).

KEY TO LIFE STAGES OF *C. OCTANGULARE*:

- 1) >8 scales per body annule (midbody), 9-113 μm ovary length, 167-300 μm body length, and 31-48 μm stylet..... 2
- 8 scales per body annule (midbody), 180-342 μm ovary length, 331-442 μm body length, and 54-60 μm stylet length..... Female
- 2) 9-46 μm ovary length, 167-257 μm body length, and 31-38 μm stylet length..... 3
- 72-113 μm ovary length, 235-300 μm body length, and 40-48 μm stylet length..... Fourth-stage juvenile
- 3) 30-46 μm ovary length, 224-257 μm body length, and 34-38 μm stylet length..... Third-stage juvenile
- 9-27 μm ovary length, 167-214 μm body length, and 31-38 μm stylet length..... Second-stage juvenile

DESCRIPTION OF STAGES

Egg: A developing second-stage juvenile was observed within an egg in an adult female. The intra-uterine egg was 70 x 24 μm and appeared to have small rounded projections on the shell (Fig. 2-A). These were arranged in rows on the surface of the egg. The projections or scales were small, about 1 μm in length and width. The probable first-stage juvenile (since no extra

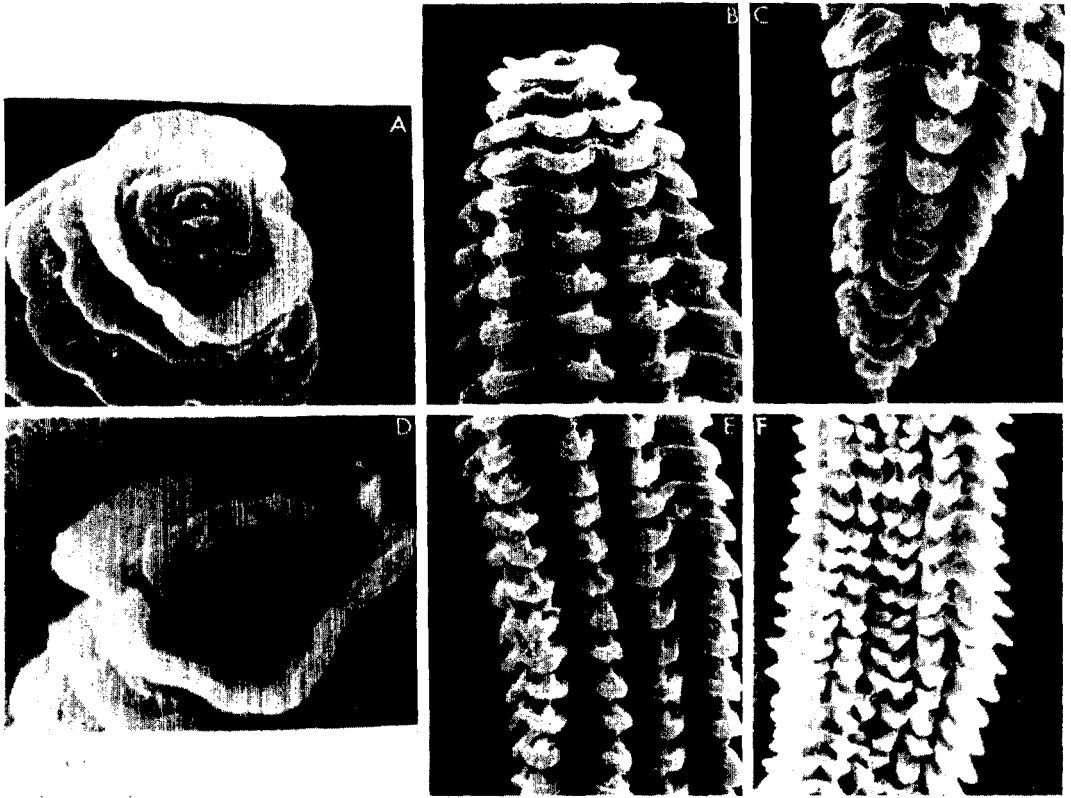


FIG. 1-(A-F). Scanning-electron micrographs of *C. octangulare*. A-E Female. A) *En face* view showing labial disc, partially extruded stylet, submedian lobes, and head annules, 2440X. B) Anterior portion of body showing scales on annules, 1268X. C) Posterior section of body showing vulva and tail terminus, 1268X. D) *En face* section showing amphids, elevated labial disc and submedian lobes, 4225X. E) Section of midbody showing arrangement of annules and scales, 1268X. F) Midbody section of second-stage juvenile showing number and arrangement of scales on annules, 1268X.

cuticle was observed within the egg) possessed a well developed stylet, 28 μm long, an anterior portion measuring 20 μm , and stylet knobs 7 μm wide. The genital primordium was oval, with 2 cell nuclei, and was about 5 μm long.

Second-stage juvenile: The body is fusiform, with 75 annules and 12 or more longitudinal rows of scales on each annule (Fig. 1-F, 2-B, J). The head consists of two nonretorse annules, with the second annule much wider than the first (Fig. 2-B, C). The stylet is strong, with forward-projecting knobs. The genital primordium in the early second stage is oval, about 9 μm long; in late second stage, this lengthens in the developing gonad to about 27 μm . The anus is not seen. The tail is rounded conoid, tapering evenly to a terminal knob as seen in adult female.

In molting, the second-stage juvenile cuticle length had a range of 171-211 μm ;

the gonad in developing third-stage juveniles in second-stage cuticle ranged from 16-27 μm (Fig. 2-C). Stylet length 33-37 μm ; anterior portions of stylet 27 μm .

Third-stage juvenile: Body fusiform, with 74 annules and 12 or more longitudinal rows of scales on each annule as in first stage. Head annules same as in second-stage juvenile. Stylet strong, with anchor-shaped knobs. Gonad about 37 μm long, and no change in tail region from that of second stage (Fig. 2-D).

Juveniles undergoing third molt have third-stage cuticle lengths of 217-235 μm . Stylets in developing fourth-stage juveniles were 42-46 μm , with cone length 35-36 μm . Gonads lengthened from 57 to 63 μm .

Fourth-stage juvenile: A cross-section through the body shows 12 longitudinal rows of scales on the annules (Fig. 2-F, I). Head shape remains unchanged. In this stage, the gonad is 86 μm long and more

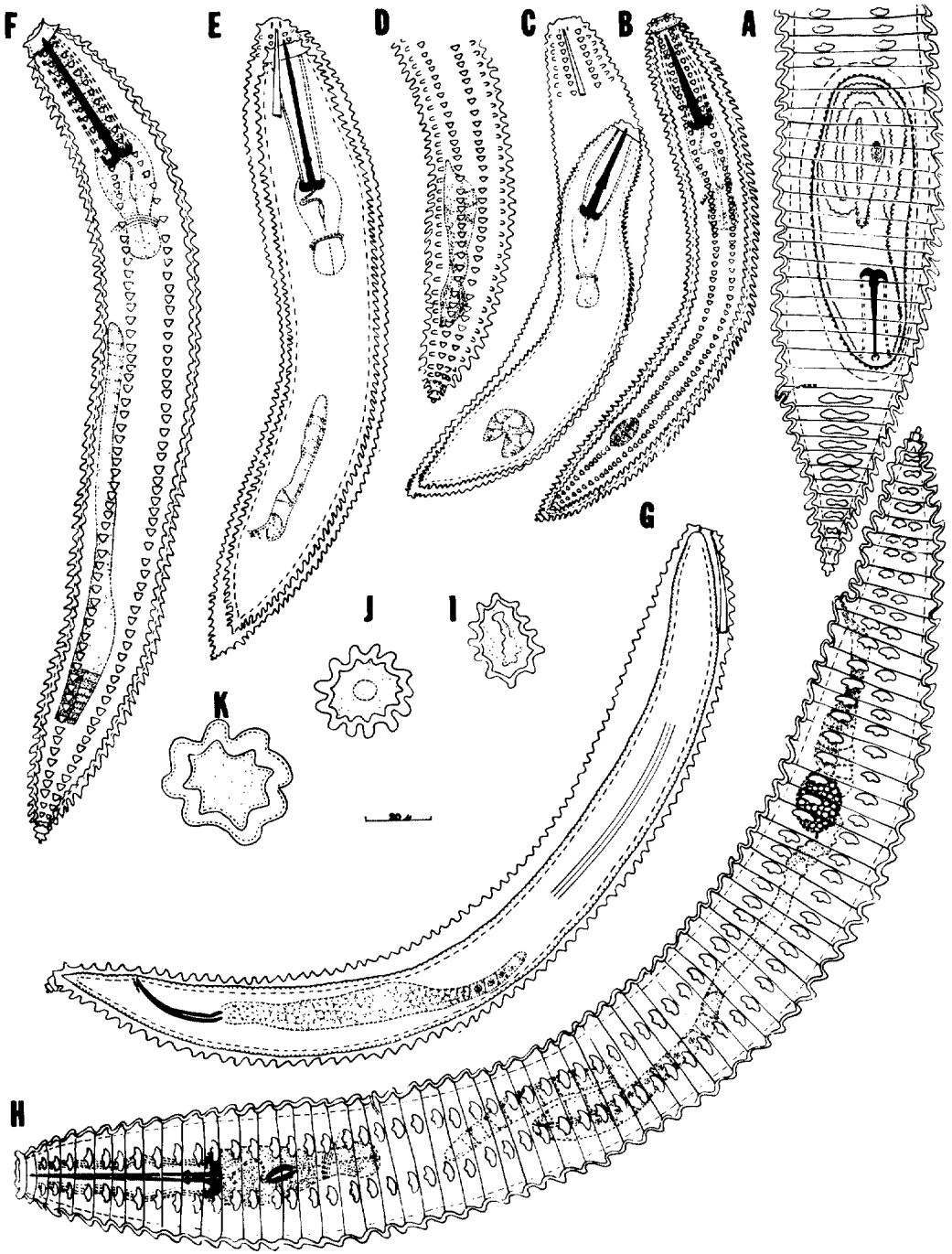


FIG. 2-A-K. Life stages of *C. octangulare*. A) 1st-stage larva in egg in posterior section of adult female. B) 2nd-stage larva. C) 2nd molt with developing 3rd-stage larva. D) Posterior of 3rd-stage larva. E) Third molt with developing 4th-stage larva. F) 4th-stage larva. G) 4th molt with developing adult male. H) Adult female. I) Cross-section through midbody of 2nd-stage larva showing 13 rows of scales. J) Cross-section through midbody of 4th-stage larva showing 12 rows of scales. K) Cross-section through midbody of adult female showing 8 rows of scales.

developed than in third stage. Tail remains the same as in the third stage.

Some fourth molts contained males (Fig. 2-G). The fourth cuticle averaged 275 μm long, with 73-75 annules. Annule width of male at midbody measured 1.6 μm . Spicules measured 29-31 μm ; gubernaculum about 6 μm . The testis was clearly defined.

Female: Body stout, fusiform (Fig. 2-H). Head truncate, with two annules, the first discoid, and set off by a constriction from the second annule, which is wider and rounded. De Grisse (1) classified the genus (*Criconema*) without submedian lobes. Mehta and Raski (6) described *C. octangulare* as *Criconema (Variasquamata) octangulare* and thus placed it in a group which possesses submedian lobes. SEM (Fig. 1-A, D) shows four small, rudimentary submedian lobes present, arranged in a dorsal and ventral pair in a rectangle around the oral aperture. Annules number 67-75; each annule divided into eight scale-like plates more or less in the shape of a rounded triangle and in eight longitudinal rows (Fig. 2-K, 1-B,C,E). Stylet 57 μm long, anterior portion measuring 43 μm . Stylet knobs directed forward and measure about 10 μm across. Excretory pore at 22nd or 23rd annule and difficult to observe in many specimens. Ovary outstretched, sometimes reaching just below stylet knobs and usually with two flexures. Spermatheca oval containing round spermatozoa. Vulva conspicuous, the two lips protruding, and on 11th or 12th annule from terminus (Fig. 2-H, 1-C). Anus on 7th annule from

terminus; this can be observed on a ventral view of the tail. Tail conoid, tapering uniformly and ending in a small knob.

Males: Numerous collections containing *C. octangulare* contained males of other species of *Criconema*; however, no males resembling the description by Hopper were collected outside of the fourth cuticle. Males of *C. octangulare* thus appear to be extremely rare.

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