

Observations of All Postembryonic Stages of *Xiphinema coxi coxi* (Nematoda: Longidoridae)¹

M. R. CHO AND R. T. ROBBINS²

Abstract: Initial morphometric data and descriptions of males and the four juvenile stages of *Xiphinema coxi coxi* Tarjan, 1964 collected from soil about the roots of alfalfa (*Medicago sativa* L.) at Gainesville, Florida, and from a greenhouse microplot at Fayetteville, Arkansas, are given. Males were similar morphometrically and in shape to females and had 3-5 preanal supplements. The four juvenile stages were easily separated by differences in body size, odontostyle, and replacement odontostyle lengths. Supplemental morphometric data for females are given along with scanning electron microscope ultrastructural information. Three *X. coxi coxi* females with abnormal gonad development are reported.

Key words: alfalfa, light microscopy (LM), *Medicago sativa* L., morphology, scanning electron microscopy (SEM), ultrastructure, *Xiphinema coxi coxi*, Z-organ.

The description of *Xiphinema coxi* Tarjan, 1964 was based on females only (11). Merritt Island, Florida, was designated as the type locality. Female specimens from Key West, Florida, and Aschersleben, German Democratic Republic, were also included in the description. Sturhan's (10) report of the *X. coxi* females from Gainesville, Florida, was the only other report of this species in the United States; in it he questioned the identification of the Key West specimen as *X. coxi*.

Since the description, populations of *X. coxi* have been reported from several European countries (1,3,10,12). Sturhan (10) differentiated the subspecies *X. coxi coxi* Tarjan, 1964 and *X. coxi europaeum* Sturhan, 1984 and a new species *X. pseudocoxi* Sturhan, 1984 based on differences in number of body pores, shape of pseudo Z-organ, tail shape, and stylet length. He concluded that the populations reported from Merritt Island and Gainesville were *X. coxi coxi*, those from England and Belgium were *X. pseudocoxi*, and *X. coxi europaeum* and *X. pseudocoxi* were found in Germany and France. He included morphometric data for all juvenile stages

and males of *X. coxi europaeum* and *X. pseudocoxi* (10). Arias et al. reported the presence of *X. coxi europaeum* and *X. pseudocoxi* in Spain (1).

Saka and Siddiqi (9) reported *X. coxi* from Malawi in East Africa. Brown et al. (2) later described two closely related species—*X. malawiense* Brown, Luc & Saka, 1983 and *X. limbeense* Brown, Luc & Saka, 1983—from the sites where *X. coxi* had been reported.

The objectives of this study were to provide additional morphometric data on *X. coxi coxi* females and initial data on *X. coxi coxi* males and all juvenile stages, to improve identification of this species, and to differentiate *X. coxi coxi* from similar species. Descriptions and morphometric data on females, males, and the four juvenile stages and observations on females with light microscopy (LM) and scanning electron microscopy (SEM) are reported.

MATERIALS AND METHODS

Female specimens used in this study were from soil collected in an alfalfa (*Medicago sativa* L.) field near the University of Florida Extension Nematology Laboratory, Gainesville. After extraction of the females needed for morphometric study, the remainder of the collection was maintained on alfalfa in a Nematology Laboratory greenhouse, University of Arkansas Experimental Farm, Fayetteville. To obtain sufficient numbers of each juvenile stage

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² Graduate Assistant and Associate Professor, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

for the study, the nematodes were extracted from the culture at various intervals, selecting only males and juvenile stages needed, and returning the remaining nematodes to the culture for further maturation and increase.

Specimens were extracted from the soil by a combined gravity sieving-Baermann funnel technique. The specimens were heat relaxed in 4% formalin and processed to glycerin by a modified Seinhorst technique (8). Permanently mounted specimens were measured with an ocular micrometer and photomicrographs were made with an automatic 35-mm camera attached to a compound microscope. Females for SEM study were processed by the critical point drying method (4) and were viewed and photographed with an ISI-60 SEM operating at 30 kV. Voucher specimens have been deposited in the USDA Nematode Collection, Beltsville, Maryland.

RESULTS

Female morphology: Morphological characteristics of *X. coxi coxi* females were described well by Tarjan (11). Morphometrics of paratypes, Sturhan's Gainesville specimens, and those of this study are given in Table 1. Females of this study had a slightly longer average body length than the type population from Merritt Island (3,793.9 vs. 3,580.0 μm) and a greater a ratio (83.3 vs. 74.7). Other morphometrics including b, c, c', and V agreed closely with those of the type population. Odontostyle length was shorter than that of the type population (117.0 vs. 122.0 μm), whereas the odontophore length was almost identical (71.5 vs. 72.0 μm). The number and the positions of body pores were almost the same as Tarjan's description. Coefficients of variation (CV) were less than 10% for most morphometric characters (Table 1). CV greater than 10% indicating relatively

higher variabilities were encountered for the b ratio (13.5%), distance from anterior end to guide ring (10.1%), and the hyaline length of tail (11.2%).

SEM study of face views showed the labial region had 16 papilliform cephalic sensory papillae (sensilla) in the normal 6–10 arrangement of Adenophorea (7). The six inner sensilla were arranged in a circle and slightly elevated (Fig. 1A, D). The 10 outer sensilla were smaller and flatter than the inner sensilla (Fig. 1D, F). The labial region was distinctively separated from the rest of the body by the slit-like amphidial opening (8–10 μm), which occupied about 60% of the corresponding lip diameter (14–16 μm) (Figs. 1A, D, 2A).

The cuticle was finely annulated with ca. 0.5- μm -wide transverse striae (Fig. 1A, E). Body pores arranged in a row on lateral, ventral, and dorsal sides were observed in the anterior part of the body (Fig. 1A, E). The arrangement of body pores in the rest of the body was more irregular. The vulval opening was a transverse slit ca. 18 μm wide. Transverse striae were obscure around the vulval opening (Fig. 1B). The tail was dorsally convex-conoid, with a ventrally positioned digitate terminus (Fig. 1C). Transverse striae were observed in the tail region, except the digitate terminus on which longitudinal ridges were evident (Fig. 1C, G).

Among the 29 gravid females observed, 10 were carrying an egg in the anterior gonad, 10 had an egg in the posterior gonad, and 9 had eggs in both gonads. A maximum number of four eggs was observed in one female, with two eggs in each gonad (Fig. 2B). One female was observed with three eggs, one anterior and two posterior. Some eggs were observed within the Z-organ, and 4–6 of the apophyses were evenly distributed around the egg (Fig. 2C, D).

Among ca. 100 females examined, three

FIG. 1. Scanning electron micrographs of *Xiphinema coxi coxi* females. A) Anterior region (arrows denote body pores). B) Vulval region. C) Tail. D) Facial view (arrows denote the positions of outer cephalic papillae). E) Body pore. F) Outer cephalic papillae and amphid, subdorsal (ventral) view. G) Tail terminus.

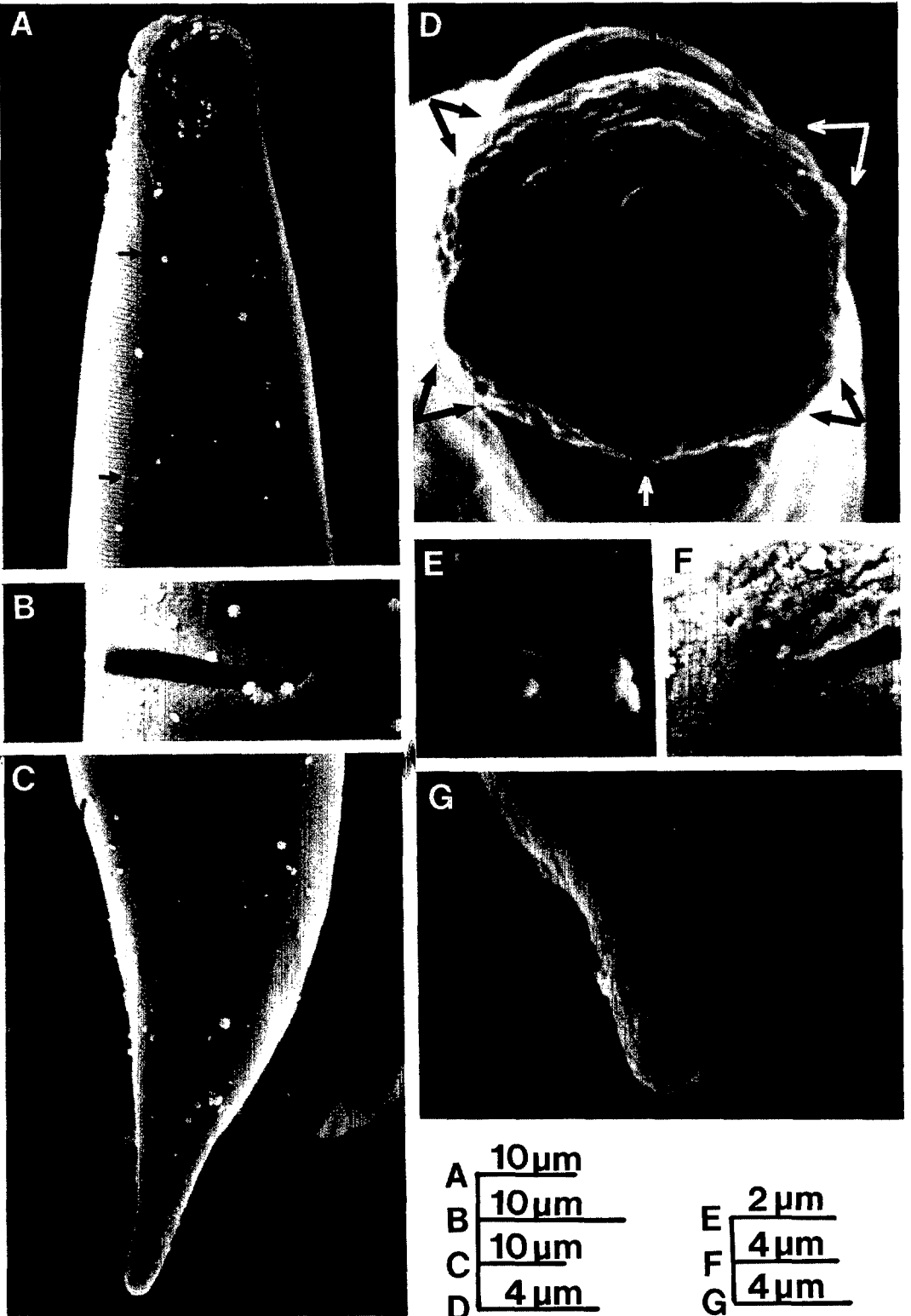


TABLE 1. Morphometric data on females and males of *X. coxi coxi* from Florida.

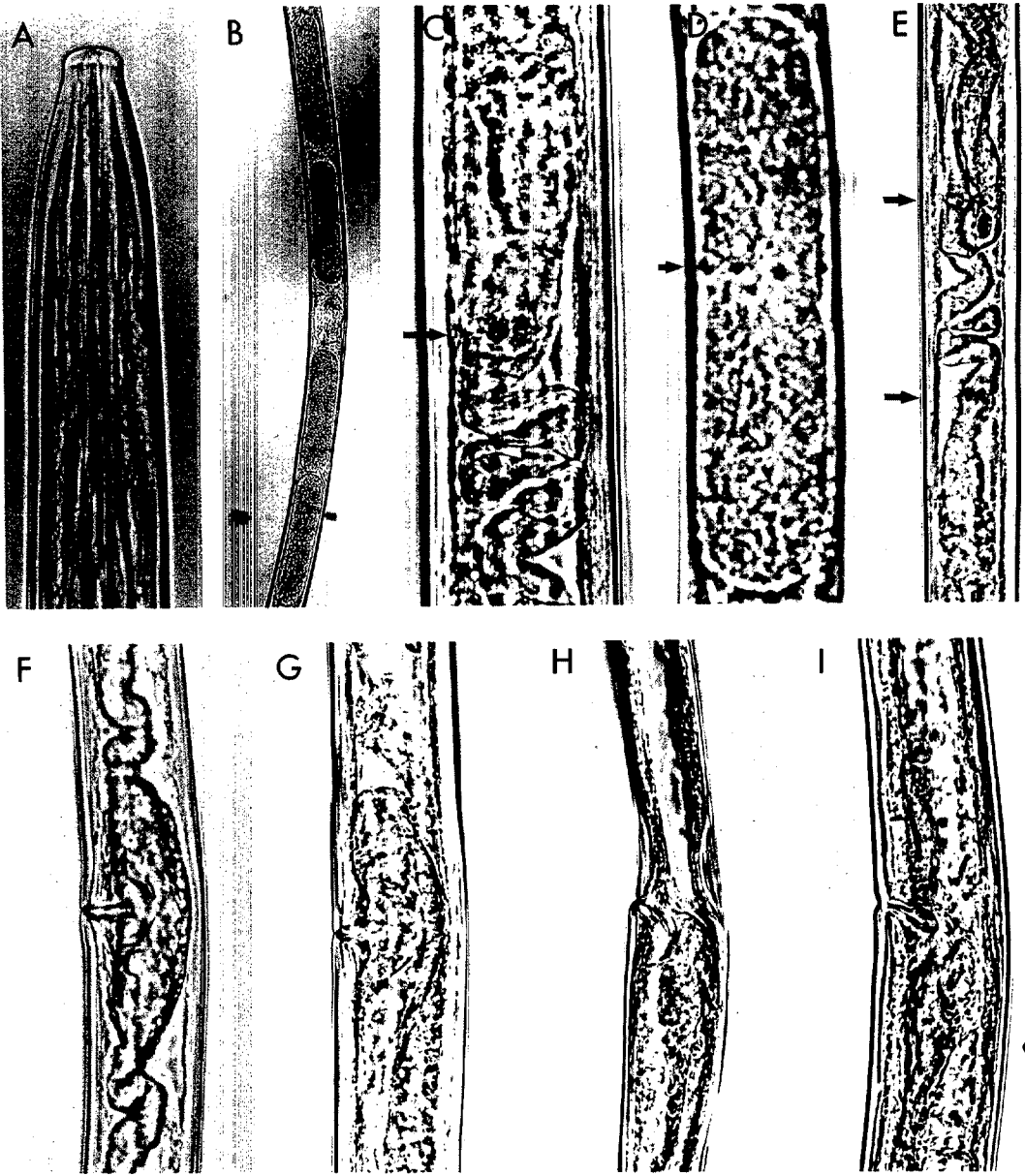
	Females, Gainesville (n = 25)†	Males, Gainesville (n = 3)‡	Females, Merritt Island (Tarjan, 1964) (n = 15)‡	Females, Gainesville (Sturhan, 1984) (n = 2)
Length (μm)	3,793.9 (217.4) 3,279–4,241 (5.7)	3,710.7 3,498–4,035	3,580.0 3,060–4,000	3,660 3,670
a	83.3 (5.0) 75.1–96.2 (6.0)	84.7 74.4–96.0	74.7 66.0–82.3	70 83
b	9.0 (1.2) 6.5–12.2 (13.5)	9.6 8.9–10.6	8.4 7.5–9.2	8.3 9.1
c	67.8 (6.3) 55.4–80.9 (9.3)	65.6 59.3–72.1	65.8 58.8–82.3	65 68
c'	1.9 (0.2) 1.7–2.2 (8.2)	1.6 1.6	1.8 1.5–2.0	1.7 1.7
V(%) (T[%])	44.9 (1.4) 41.2–47.7 (3.2)	45.7 40.0–51.3	44.0 40.0–46.0	43.2 44.6
Odontostyle (μm)	117.0 (2.8) 110–121 (2.4)	118.7 113–123	122 113–127	118 121
Odontophore (μm)	71.4 (2.0) 68–75 (2.7)	68.0 66–70	72 68–82	71 74
Stylet (μm)	188.4 (4.0) 180–194 (2.2)	186.7 179–191	194 185–210	192 193
Anterior end to guide ring (μm)	102.4 (10.4) 76–114 (10.1)	108.3 104–111		112 117
Lip width (μm)	14.2 (0.5) 14–16 (3.3)	14.3 14–15		
Esophagus base length (μm)	98.2 (11.6) 61–120 (11.8)	106 102–110		
Esophagus base width (μm)	18.0 (3.3) 12–28 (18.2)	21.3 20–22		
Tail (μm)	56.2 (4.1) 48–62 (7.4)	56.7 55–59		53 56
Anal body width (μm)	29.4 (1.1) 27–31 (3.8)	35.3 35–36		
Hyaline length (μm)	21.6 (2.4) 18–25 (11.2)	22.3 19–25		
Hyaline width (μm)	10.8 (1.0) 9–14 (9.6)	11.3 9–14		

† Mean and (standard deviation) above the range and (coefficient of variation in percentage).

‡ Mean above the range.

had abnormal gonad development. The normal didelphic, amphidelphic condition is shown in Figure 2F. The gonad of one abnormal female extended 50 μm anteriorly before reflexing posteriorly so that both Z-organs and ovaries were posterior to the vulva. The Z-organs of the reflexed and posterior ovary were observed at 160 μm and 240 μm , respectively, from the vulva (Figs. 2E, G, 3A, B). The gonads of the second abnormal female exhibited the didelphic, opisthodelphic condition with the

vagina and both uteri extending posteriorly immediately upon leaving the vulva. The Z-organs were observed at 390 μm and 470 μm from the vulva (Figs. 2H, 3C, D). The third abnormal female was monodelphic and postpudendum. In this specimen the anterior gonad was lacking, the vagina sloped posteriorly immediately upon leaving the vulva, and little of the posterior gonad was discernable. The Z-organ was observed at 740 μm from the vulva (Figs. 2I, 3E, F). The posteriorly reflexed ante-



— scale bar

FIG. 2. Micrographs of females of *Xiphinema coxi coxi*. Scale bar: 20 μm (A, C, D); 100 μm (B); 40 μm (E); 30 μm (F-I). A) Anterior region. B) Female with four eggs. C) Z-organ (arrow). D) Egg in Z-organ and apophyses (arrow). E) Abnormal female with two gonads and Z-organ (arrow) directed posteriorly. F) Vulval region of normal female. G-I) Vulval region of abnormal females.

rior gonads of the abnormal females were difficult to trace except for the Z-organs. All other observed characters were normal.

Male morphology: Morphometrics are given in Table 1. When heat killed and re-

laxed, the body formed an open "J," with the more pronounced curvature at the posterior end. The body was elongated and tapered for a short distance to the anterior end. The lip region was not set off by a constriction, but was narrower than the rest

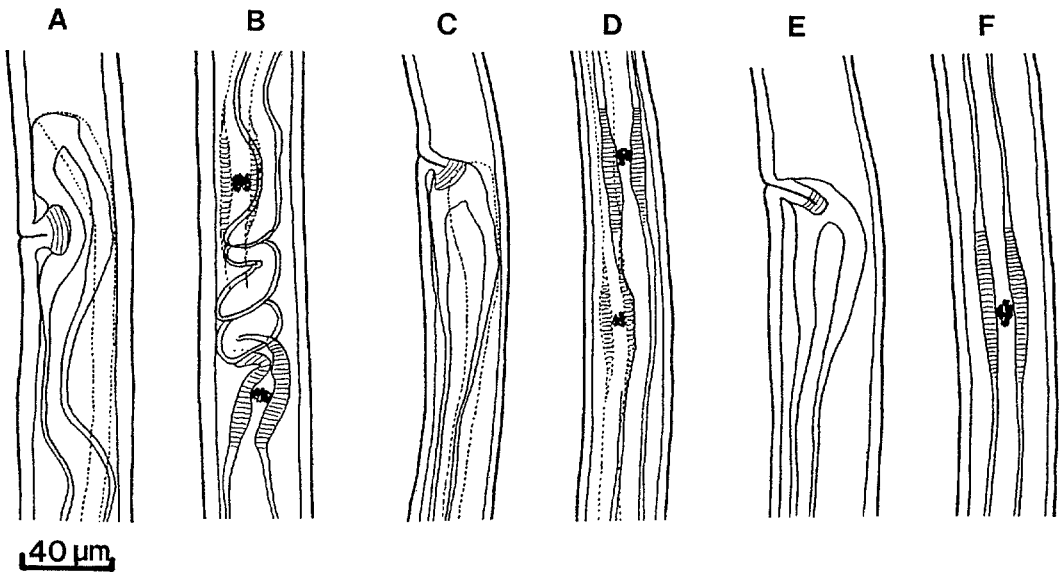


FIG. 3. Line drawings of abnormal females vulval and Z-organ regions of *Xiphinema coxi coxi*. Reflexed anterior gonads drawn with dotted lines. Figures A and B correspond to Figure 2G and E; Figures C and D correspond to Figure 2H; Figures E and F correspond to Figure 2I.

of the body. The cuticle appeared as two distinct layers and was $2\ \mu\text{m}$ thick at the esophago-intestinal junction, $2\text{--}3\ \mu\text{m}$ at midbody, and $4\text{--}5\ \mu\text{m}$ on the dorsal side of the tail. The body width was $39\text{--}44\ \mu\text{m}$ at the esophago-intestinal junction and $42\text{--}47\ \mu\text{m}$ at midbody. The amphid was $4\ \mu\text{m}$ from the anterior end, and the amphidial opening was a transverse slit of $10\ \mu\text{m}$ in length. The odontostyle and odontophore were typical for the species, with well-developed flanges, $12\text{--}13\ \mu\text{m}$ wide with reinforced margins. The guide ring tube was $7\text{--}9\ \mu\text{m}$ long. The dorsal esophageal nucleus was located $8\text{--}10\ \mu\text{m}$ from the anterior end of the bulb and had a diameter of $2.5\ \mu\text{m}$. The two subventral nuclei were

TABLE 2. Distances (μm) between supplements in males of *X. coxi coxi*.

Specimen no.	Cloaca to PS†	PS-S1	S1-S2	S2-S3	S3-S4
1	20	78	38	34	43‡
2	18	90	34	14	
3	14	96	46		

† PS = paired preanal supplement.

‡ Rudimentary supplement.

$49\text{--}51\%$ from the anterior end of bulb and had diameters of $1\text{--}1.5\ \mu\text{m}$. A $3\text{-}\mu\text{m}$ -long mucro was observed $26\ \mu\text{m}$ from the base of the odontophore in the lumen wall of one specimen. The hemizonid was flat, $2.3\text{--}3.5\ \mu\text{m}$ long, $194\text{--}206\ \mu\text{m}$ from the anterior end. The nerve ring was $14\text{--}21\ \mu\text{m}$ wide, $22\text{--}38\ \mu\text{m}$ from the base of the odontophore. Spicules were $50\text{--}54\ \mu\text{m}$ long, with a $17\text{--}22\text{-}\mu\text{m}$ -long accessory piece. Paired subventral supplements were located just anterior to the cloaca; anteriorly there were $2\text{--}4$ single ventral supplements (Table 2). Three caudal pores were observed; one subventral adjacent to the spicules, one subdorsal at the level of the anal opening, and the other lateral near the point where the tail peg begins. The tail was ventrally digitate, dorsally convex-conoid with a $17\text{--}22\text{-}\mu\text{m}$ -long peg (Fig. 4F-I).

Juvenile morphology: Morphometrics are given in Table 3. All stages formed an open "C" when heat killed and relaxed, lying much straighter than adults. The four juvenile stages were clearly separated from each other and from adults by length of body, odontostyle, and replacement odontostyle (Fig. 5). Length of the odontophore

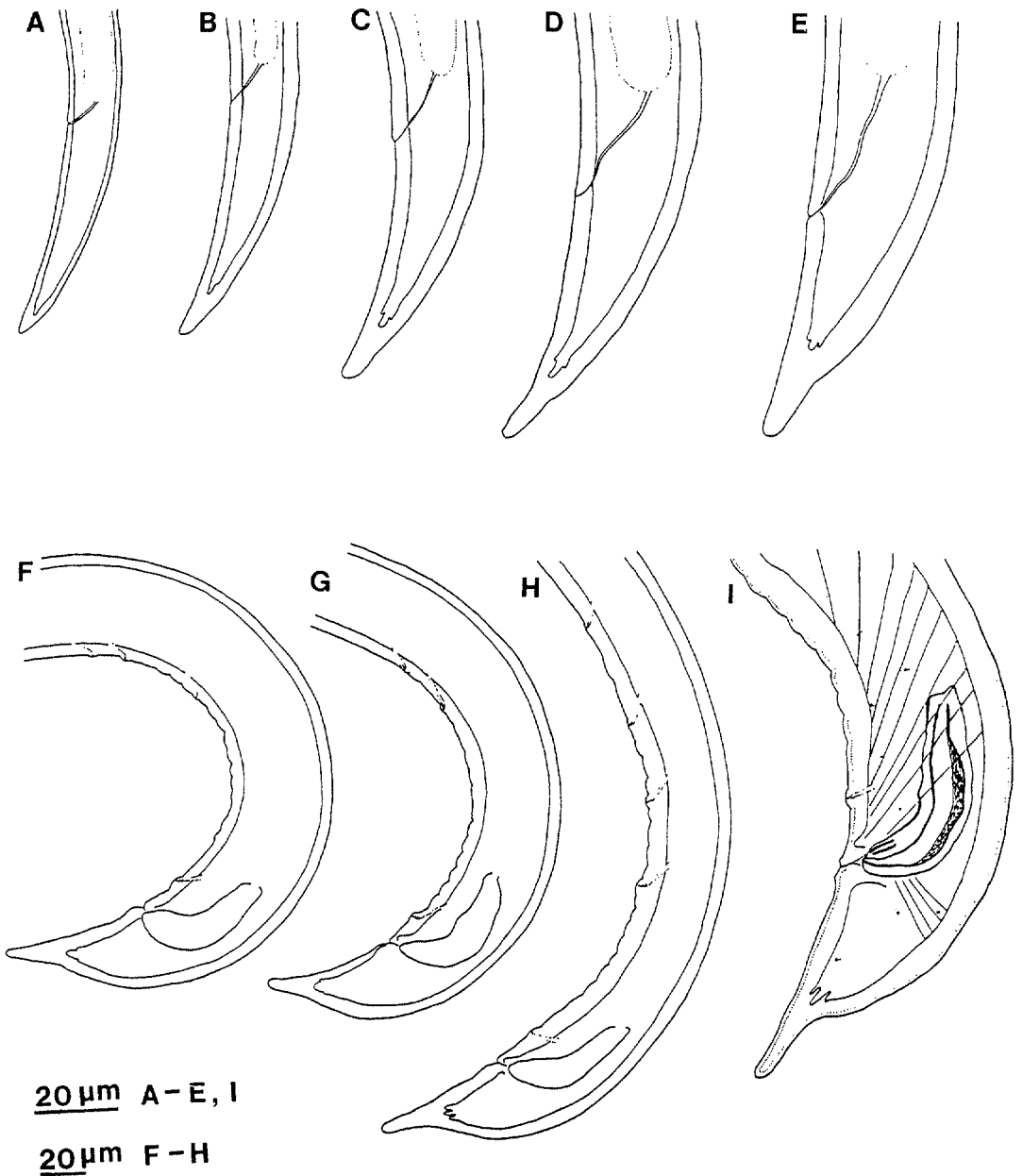


FIG. 4. Tail shapes of four juvenile stages, female, and males of *Xiphinema coxi coxi*. A) First stage. B) Second stage. C) Third stage. D) Fourth stage. E) Female. F-I) Males.

increased with each stage; however, there was some overlap between the stages. Relative values of a , b , and c increased with each stage, whereas the c' value decreased. J1 tails were slightly shorter than those of J2, J3, and J4 stages (average 56.5 vs. 63.8, 64.0, and 64.2 μm). Tails of the J2 through J4 were almost equal in length, and in all stages are elongate-conoid and slightly

curved ventrally. Three caudal pores were observed in all stages of juveniles. Length and width of the hyaline region increased with each stage (Fig. 4A-D).

DISCUSSION

Since the original description of *X. coxi* by Tarjan in 1964 (11), several new species and one subspecies, comprising the *X. coxi*

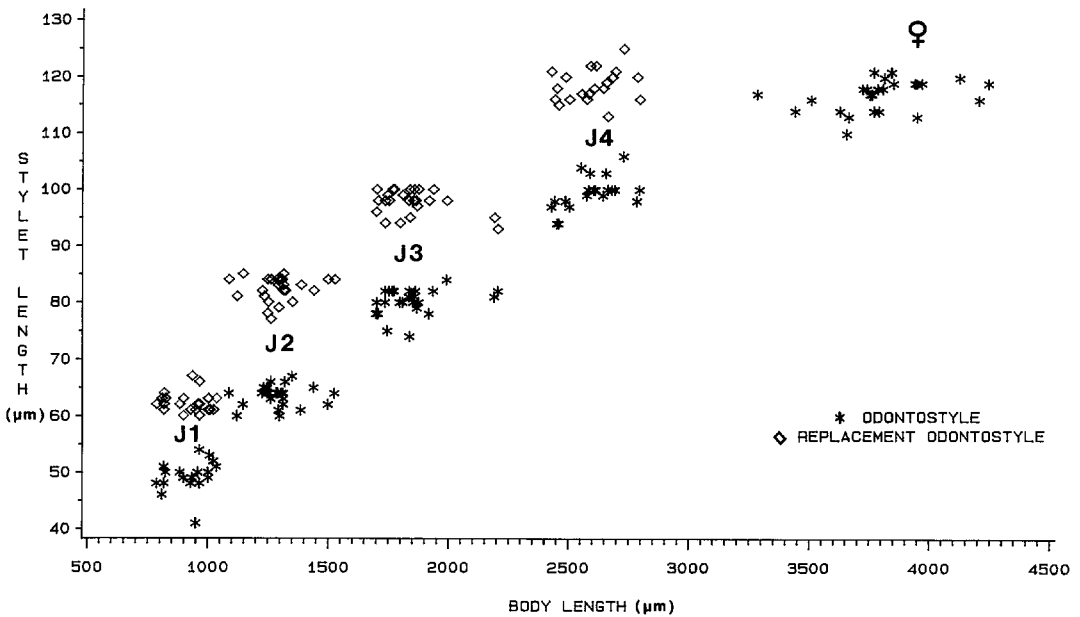


FIG. 5. Relationship between body, odontostyle, and replacement odontostyle lengths of the four juvenile stages and females of *Xiphinema coxi coxi*.

group, have been described (2,10). Species in the *X. coxi* group are morphologically similar to each other and differences among them are indistinct. Among all the characters used in differentiation of the species in the *X. coxi* complex, the Z-organ structure is the most distinguishable. The 4–5 apophyses of the *X. coxi coxi* are more opaque (denser, sclerotized) than in the other species in the *X. coxi* complex which all contain a weakly developed lumen wall and numerous bubble-like globules. There are no fundamental differences or a clear-cut distinction between a “typical” and a “pseudo” Z-organ; however, the Z-differentiation is useful for specific determination (5). The name of the Z-differentiation in *X. coxi* sensu Tarjan has been inconsistent among the authors (2,5,10,11). The structure of the Z-differentiation observed in *X. coxi coxi* seems closer to the “typical” Z-organ, whereas those of the other species in the complex would belong to the “pseudo” Z-organ, according to the definitions proposed (5).

Only six males have been described within the complex; one *X. pseudocoxi*, two *X. coxi europaeum*, and the three *X. coxi coxi* of

this study. There are three caudal pores in *X. coxi coxi* males, whereas *X. coxi europaeum* and *X. pseudocoxi* males have four and four or five, respectively. Spicules of *X. coxi coxi* males are slightly shorter than those of *X. coxi europaeum* (50–54 vs. 58–60 μm) and slightly longer than in *X. pseudocoxi* (48 μm). Supplement numbers are similar for all the species of the complex.

Morphometric comparison of the juvenile stages among *X. coxi coxi*, *X. coxi europaeum*, and *X. pseudocoxi* indicate tail lengths are the only significant difference. The tail lengths of *X. coxi coxi* are stable in J2, J3, and J4 stages before being reduced in the adult stage; however, the lengths continuously increase in *X. pseudocoxi* from J1 through J4 (48, 56, 68, and 73 μm , respective averages).

Correct identification of the species are emphasized because of their importance as virus vectors (10). Males are very rare or unknown among the species in the *X. coxi* complex, which suggests that parthenogenesis is the mode of reproduction. The taxonomic problems of thelytokous species have been well discussed, and the usefulness of canonical variate analysis in helping

TABLE 3. Morphometric data on four juvenile stages of *X. coxi coxi* from Gainesville, Florida.

	J1 (n = 22)	J2 (n = 24)	J3 (n = 24)	J4 (n = 20)
Length (μm)	920.3 (79.9) 785-1,033 (8.7)	1,289.7 (102.9) 1,084-1,524 (8.0)	1,841.3 (133.3) 1,694-2,202 (7.2)	2,592.3 (111.6) 2,423-2,792 (4.3)
a	40.8 (2.0) 37.3-45.9 (4.9)	43.4 (6.6) 33.1-56.7 (15.3)	57.1 (5.9) 43.6-68.8 (10.3)	74.2 (7.6) 61.5-88.0 (10.2)
b	4.6 (0.7) 3.6-5.8 (14.4)	4.7 (0.6) 3.3-5.8 (13.9)	5.2 (0.6) 4.0-7.0 (11.5)	6.7 (0.6) 5.5-7.7 (8.3)
c	16.3 (1.6) 13.8-20.0 (9.8)	20.2 (1.6) 18.4-22.5 (5.7)	28.9 (0.3) 24.9-35.5 (8.7)	40.5 (3.1) 35.2-47.3 (7.7)
c'	3.9 (0.4) 3.1-5.1 (11.5)	3.6 (0.3) 3.0-4.4 (9.1)	3.0 (0.3) 2.0-3.4 (10.2)	2.4 (0.2) 2.1-2.8 (7.3)
Odontostyle (μm)	49.3 (2.6) 41-54 (5.3)	63.7 (1.8) 60-67 (2.9)	80.2 (2.3) 74-84 (2.9)	99.5 (3.0) 94-106 (3.0)
Odontophore (μm)	39.1 (2.3) 35-44 (6.0)	47.1 (2.0) 41-50 (4.3)	55.6 (2.8) 52-64 (5.1)	63.4 (1.4) 60-66 (2.3)
Styilet (μm)	88.4 (3.3) 79-94 (3.7)	110.8 (2.5) 106-116 (2.3)	135.8 (3.9) 128-145 (2.9)	162.9 (3.5) 154-170 (2.2)
Replacement odontostyle (μm)	62.3 (1.7) 60-67 (2.8)	82.3 (2.2) 77-85 (2.7)	97.8 (2.2) 93-100 (2.2)	118.6 (2.8) 113-125 (2.4)
Guide ring (μm)	40.0 (2.4) 35-44 (6.1)	54.5 (4.5) 47-65 (8.2)	69.0 (7.1) 53-86 (10.3)	83.7 (8.2) 63-96 (9.7)
Lip width (μm)	9.0 (0.2) 8-9 (2.4)	9.9 (0.6) 9-12 (5.9)	11.1 (0.5) 10-12 (4.8)	12.5 (0.6) 12-14 (4.9)
Esophagus base length (μm)	55.1 (6.9) 42-68 (12.6)	67.4 (5.8) 60-88 (8.5)	78.0 (6.0) 64-88 (7.7)	90.2 (5.9) 80-102 (6.5)
Esophagus base width (μm)	12.3 (1.5) 9-14 (12.1)	15.6 (2.4) 12-20 (15.2)	17.0 (2.2) 12-23 (12.7)	16.9 (2.8) 12-21 (16.4)
Tail (μm)	56.5 (3.7) 50-64 (6.5)	63.8 (4.6) 56-75 (7.2)	64.0 (4.3) 53-75 (6.7)	64.2 (4.1) 59-75 (6.3)
Anal body width (μm)	14.6 (1.7) 11-17 (11.5)	18.0 (1.7) 15-22 (9.7)	21.7 (1.7) 19-26 (8.0)	26.8 (1.4) 25-31 (5.2)
Hyaline length (μm)	8.2 (1.0) 5-9 (11.8)	12.5 (2.0) 8-16 (15.8)	15.2 (1.5) 13-20 (10.1)	18.2 (1.9) 14-22 (10.6)
Hyaline width (μm)	4.8 (0.4) 4-5 (9.0)	6.0 (0.4) 5-7 (6.0)	7.1 (0.9) 6-9 (12.6)	8.1 (0.6) 7-9 (7.5)

Mean and (standard deviation) above the range and (coefficient of variation in percentage).

to define the limits of several species was demonstrated (6). As suggested by Brown et al. (2), an in-depth taxonomic comparison of *X. basiri*, *X. coxi*, *X. malawiense*, *X. limbeense*, and *X. pseudocoxi* would be useful. These species already have been compared with *X. pseudocoxi* (7). The details and information from this report are intended to improve the accuracy of identification of *X. coxi coxi* and clarify its relationship to related species.

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