Anguina plantaginis n. sp. Parasitic on Plantago aristata with a Description of Its Developmental Stages[1]

HEDWIG HIRSCHMANN[2]

Abstract: Anguina plantaginis n. sp., parasitic on *Plantago aristata*, is described and illustrated. This new species is most closely related to *A. klebahni*, *A. millefolii*, *A. mobilis*, and *A. moxae* and is characterized as follows: moderate body size for the genus; absence of esophageal "storage organ"; postvulval uterine sac extending about 45% of vulva-anus distance; crustaformeria of young females longer than spermatotheca or uterus proper; spicules with 2 sclerotized thickenings; long, conical tail in both sexes, narrowing at about 1/6 of its length to peg-like tip; parasitic only on *P. aristata*. Two nematode generations that are morphologically similar but differ in body size develop in one plant gall. The postembryogenesis, studied with respect to morphological development of the larval stages, is similar to that of *Ditylenchus*. The sexes can be differentiated from the second molt on. The infective larva is the third stage, which is morphologically distinct from the regularly developing third-stage larva. *Key Words:* postembryogenesis, morphology.

In general, *Anguina* species are known to have narrow host ranges. Among the 26 species described to date, most are obligate parasites on monocotyledonous plants, mainly of the Gramineae. Some species, however, infect dicotyledonous plants including Compositae, Primulaceae, and Polygonaceae. These species are: *A. balsamophila* (Thorne) from *Balsamorrhiza sagittata* Nutt. and *Wyethia amplexicaulis* Nutt.; *A. moxae* Yokoo and Choi from *Artemisia asiatica* Nakai; *A. millefolii* (Löw) from *Achillea millefolium* L.; *A. mobilis* Chit and Fisher from *Arctotheca calendula* (L.); *A. chartolepidis* Poghossian from *Chartolepis bickersteini* J. and Sp.; *A. klebahni* Goffart from *Primula florindae*

Received for publication 3 February 1977.

[1] Paper No. 5180 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh, N.C. 27607.

[2] Department of Plant Pathology, North Carolina State University, Raleigh 27607. Sincere appreciation is extended to Dr. F. G. Giesbrecht, Department of Statistics, for statistical analyses and to Karen McKee, Department of Plant Pathology, for assisting with the illustrations.

Ward.; and *A. polygoni* Poghossian from *Polygonum alpestre* C.A.M. Species occurring on dicotylendonous plants, however, may not be closely related since they infect plants of widely differing groups.

This paper deals with the description and postembryogenesis of a new species, *A. plantaginis,* that causes formation of galls on leaves, peduncles, and inflorescences of *Plantago aristata* Michx., a member of the Plantaginaceae. Biological observations of two populations of this nematode have been reported (14). This species exhibits a high degree of parasitic specialization as only *P. aristata* was parasitized.

MATERIALS AND METHODS

Anguina plantaginis, collected from the type locality in North Carolina, was propagated in the greenhouse on *Plantago aristata at* 25 to 30 C. Seeding nematode galls and healthy Plantago seeds side by side in rows in a steam-sterilized 3:1 soil-sand mixture insured highest number of leaf infections.

Galls were dissected in water, and nematodes of all stages were selected, observed alive, or killed by gentle heat and mounted in water or 1% formalin, or stained with 1% acetic orcein (8) for morphologic study. Permanent mounts were made from specimens fixed in 2% formalin and transferred to glycerin by Seinhorst's rapid method (11). Measurements, including those of eggs, were made from specimens mounted in 1% formalin. Individuals selected for morphologic study and measurement were taken from a large series of different galls at different times of the year.

SPECIES DESCRIPTION

Anguina plantaginis n. sp.

Two generations of this *Anguina* species that differ in body size usually develop in one plant gall. Adults of the first generation are fewer but larger than those of the second generation. One gall may contain 10-15 large individuals of the first generation which are at the end of their life span, 60 young females and 50 males of smaller size, and large numbers of all other larval stages including eggs and infective larvae. Both sexes are nearly equally represented in most galls, although the few nematodes present in small galls may be of the same sex. Except for distinct differences in size, adults of the two generations are morphologically similar. The description is based on first-generation adults and is supplemented by information about differences in specimens of the second generation.

FEMALES:

Measurements—First generation (n = 40): Body length: 1,187.5-2,062.5 μm (mean 1,528.3 μm, standard deviation $\pm$ 184.0); body width: 38.0-71.4 μm (54.6 μm $\pm$ 9.1); stylet length: 9.8-10.6 μm (10.3 μm $\pm$ 0.12); dorsal esophageal gland orifice: 0.9-1.3 μm (1.01 μm $\pm$ 0.12); esophagus length: 153.7-251.8 μm (200.1 μm $\pm$ 21.4); excretory pore: 117.0-208.3 μm (149.8 μm $\pm$ 18.4); vulva anus distance: 86.4-195.0 μm (133.2 μm $\pm$ 23.2); width at vulva: 32.1-53.0 μm (40.1 μm $\pm$ 4.5); postvulval uterine sac length: 45.1-93.2 μm (61.8 μm $\pm$ 10.9); anal body diameter: 15.1-21.4 μm (18.7 μm $\pm$ 1.5); tail length: 55.5-86.4 μm (73.7 μm $\pm$ 7.0); a: 22.1-42.5 (28.4 $\pm$ 3.7); b: 5.9-8.8 (7.7 $\pm$ 0.74); c: 14.9-25.8 (20.8 $\pm$ 2.2); c': 3.1-4.6 (3.7 $\pm$ 0.37); excretory pore %: 8.3-14.4 (9.9 $\pm$ 1.2); V: $^{56.6\text{-}92.5}$ (77.4 ± 8.9) 81.6-90.0 (86.5 ± 1.6) $^{2.6\text{-}5.1}$ (4.1 ± 0.55).

Second generation (n = 45): Body length: 925.0-1,415.0 μm (1,268.1 μm $\pm$ 94.8); body width: 35.2-54.4 μm (41.3 μm $\pm$ 4.6); stylet length: 9.2-10.2 μm (9.9 μm $\pm$ 0.32); dorsal esophageal gland orifice: 0.9-1.4 μm (1.05 μm $\pm$ 0.14); esophagus length: 167.0-228.7 μm (200.8 μm $\pm$ 18.3); excretory pore: 122.0-160.2 μm (143.8 μm $\pm$ 8.7); vulva anus distance: 89.3-160.2 μm (123.9 μm $\pm$ 16.3); width at vulva: 33.3-39.5 μm (35.8 μm $\pm$ 1.8); postvulval uterine sac length: 30.6-62.4 μm (49.8 μm $\pm$ 5.8); anal body diameter: 17.4-19.3 μm (18.3 μm $\pm$ 0.62); tail length: 51.0-81.6 μm (70.6 μm $\pm$ 7.1); a: 22.0-37.7 (31.0 $\pm$ 3.5); b: 5.4-8.1 (6.4 $\pm$ 0.64); c: 14.3-23.6 (18.1 $\pm$ 1.8); c': 3.7-4.5 (4.1 $\pm$ 0.28); excretory pore %: 10.1-13.8 (11.4 $\pm$ 0.70); V: $^{56.8\text{-}76.6}$ (66.1 ± 4.1) 77.2-89.2 (84.5 ± 1.9) $^{3.2\text{-}5.6}$ (3.9 ± 0.46).

Description (Fig. 1, 2)—Body slightly curved ventrally when killed by gentle

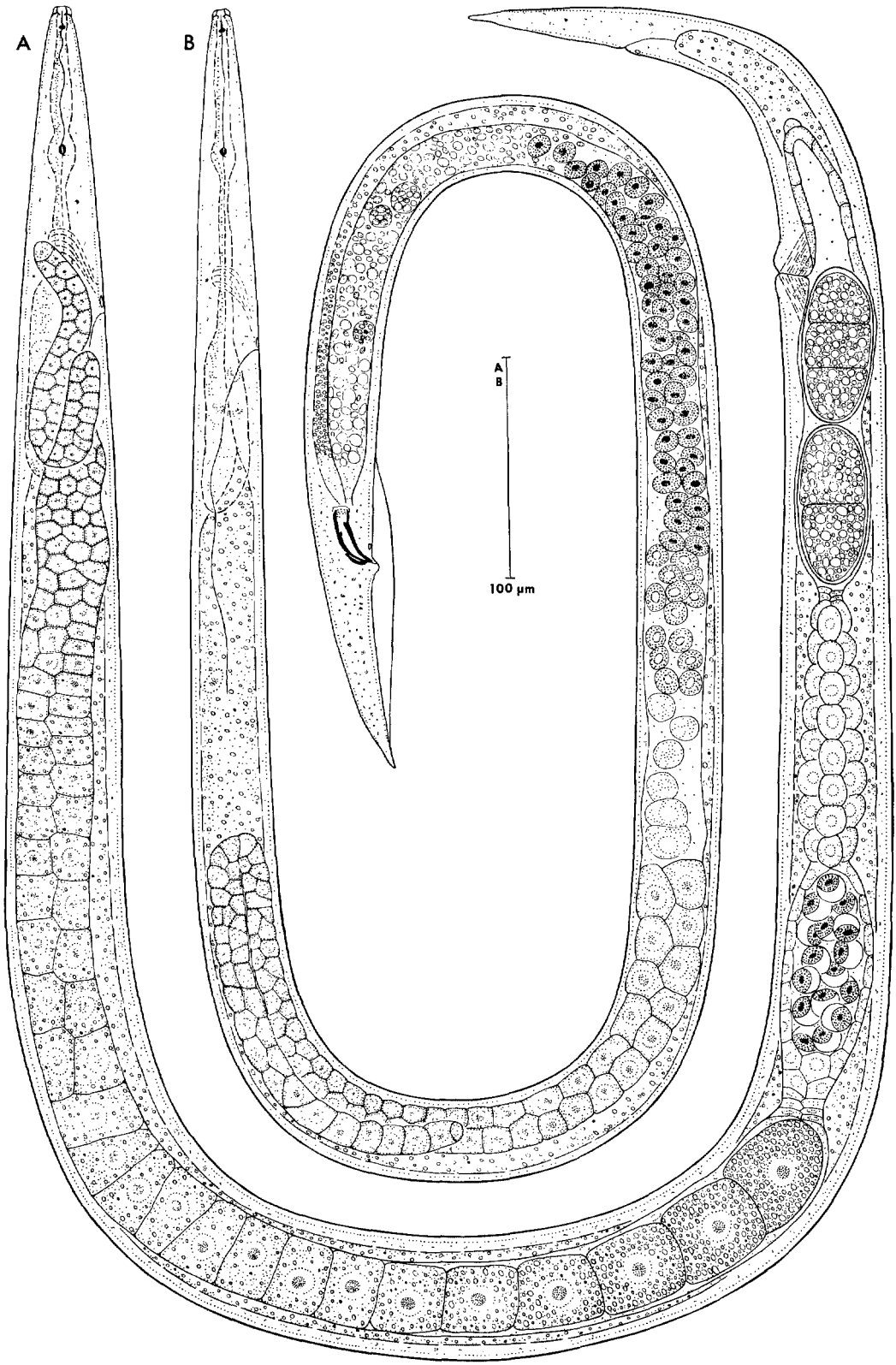

FIG. 1-(A, B). Adults of *Anguina plantaginis* n.sp. *A)* Full-length outline (lateral) of female. *B)* Full-length outline (lateral) of male. Curvature of specimens not representative of actual position assumed when heat-relaxed.

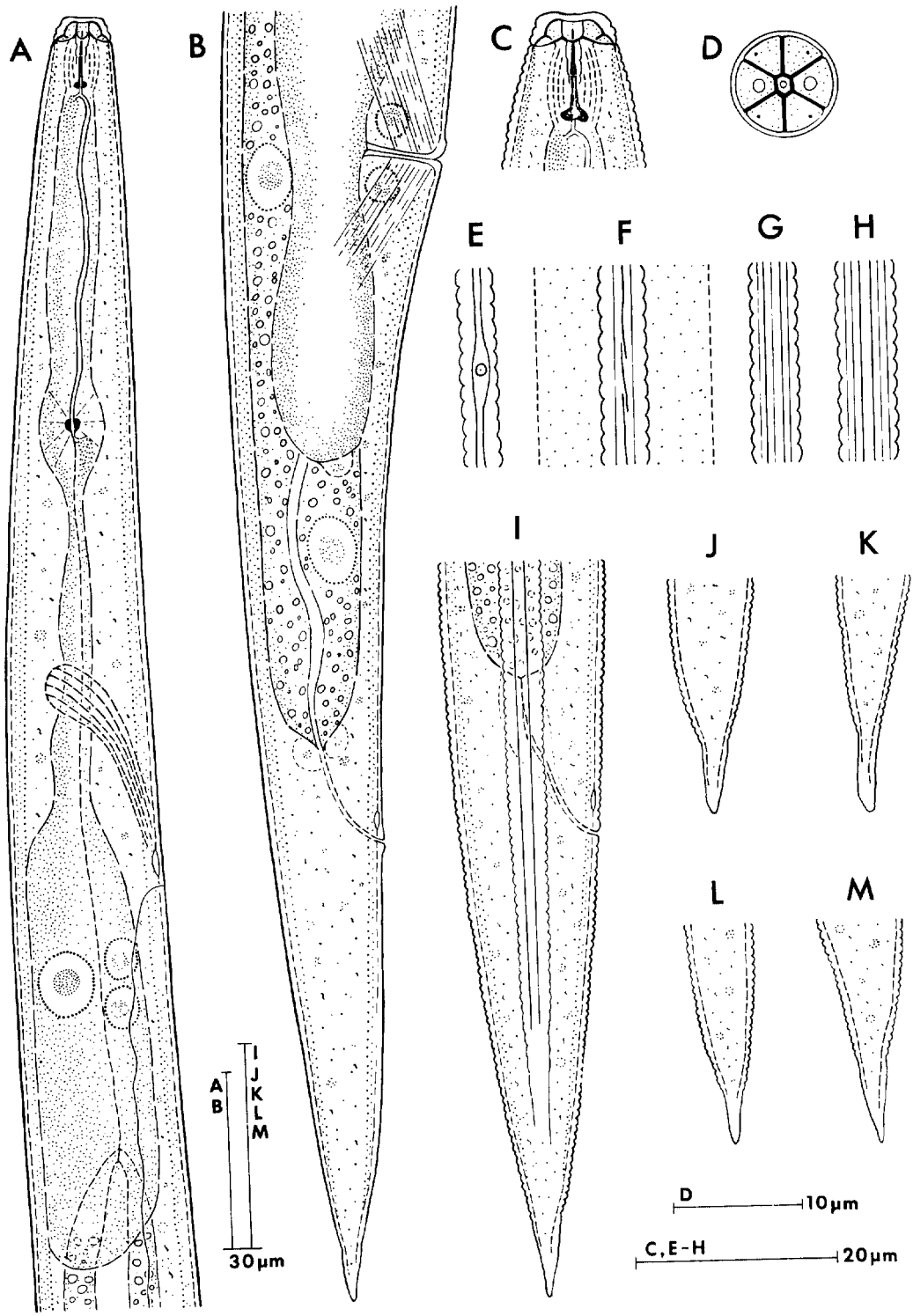

FIG. 2-(A-M). Females of *Anguina plantaginis* n.sp. *A)* Esophagus region (lateral). *B)* Posterior body region including vulva and postvulval uterine sac (lateral). *C)* Cephalic region (lateral). *D)* Face view at level of basal plate of framework. *E-H)* Variation in lateral field. *I)* Tail (lateral). *J-M)* Variation in shape of tail tip.

heat; not spirally coiled; tail bent back, sometimes vulva facing outside; obese, first-generation females largely immobile but not as plump as those of other members of genus; second-generation females especially are actively moving, resemble *Ditylenchus*. Cuticle thin, finely annulated except for very end of peg-like tail tip which is smooth. Subcuticle doubly striated. In most specimens, lateral fields at midbody basically consist of 4 distinct incisures with 3 finer incisures spaced inbetween. In some females, however, there are as many as 8 or 9 incisures in certain body parts. In anterior and posterior region, there are usually 4 incisures. Lateral field difficult to see in vulval region of obese specimens; sometimes areolated in tail region. Width of lateral field at midbody ranging from 4.1-6.1 μm (5.1 μm $\pm$ 0.48, n = 15). Underlying lateral chords very broad, at least 4 times as wide as lateral field. Deirids at level of excretory pore, very distinct in young specimens. Phasmids not seen. Somatic musculature appears degenerate in large females of first generation. Lip region with 2-3 annules, slightly set off and flattened in front. Framework weakly developed, extending slightly into body. Amphidial pouches distinct. Short stylet with stout shaft and slightly backward sloping knobs; conical part very thin. Guiding ring appears double. Dorsal esophageal gland orifice 0.9-1.4 μm behind stylet knob base. Esophagus with wide procorpus filled with secretion granules; oval median bulb with well-developed valve plates slightly anterior to middle; isthmus slightly swollen posteriad. Esophageal glands enclosed in bulbous swelling which overlaps intestine dorsally and laterally. Overlap pronounced in large females of first generation, less developed in smaller females of second generation so that glands almost abut intestine. Dorsal gland nucleus large; subventral nuclei smaller and less distinct. No "storage organ" observed. Esophago-intestinal junction very distinct in young females, obscured in older specimens. Esophageal lumen well cuticularized up to valve of metacorpus; less distinct posteriad and passes through glands toward ventral side. Nerve ring encircles isthmus slightly behind middle. Excretory pore near level of beginning of basal gland lobe in young females. Excretory duct extends back 1.5 to 2 times length of gland lobe. Excretory canal in right lateral chord with large sinus nucleus near terminus of duct, 158.1-214.2 μm (180.8 μm $\pm$ 18.0; n = 20) posteriad to excretory pore. Hemizonid 4 annules wide; approximately 2 annules in front of excretory pore. Intestinal cells very large; contain granular cytoplasm and large nuclei; apparently only 2 cells in circumference; lumen zig-zag. No postanal diverticulum present. Gonad single, directed anteriad. Vulva located much posteriorly, a very broad slit with sometimes prominent, round, protruding lips, without cuticular flaps. Postvulval uterine sac about 1.5 times the body width at vulva (43% of vulval-anus distance), lined with flat epithelium, usually terminating in distinctly set-off tip containing one or two nuclei. In large females of first generation, gonad extends to level of median bulb. Ovary lined with epithelium and cap cell at apex, usually reflexed twice in females of first generation. Germinal zone with several rows of oogonia; anterior part of growth zone with multiple rows of oocytes that decrease in number posteriorly; oocytes arranged around a rachis; posterior growth zone with large oocytes in single file; epithelium multinucleate and thicker in posteriormost part of ovary. Unfertilized oocytes in this gonad part round up but remain in single file. This region of female gonad may correspond to enlarged region anteriad of spermatotheca as depicted in *A. tritici* (Steinbuch) (13; Fig. 1-A). In *A. plantaginis,* however, oocytes do not advance in multiple rows but remain in single file. Ovary followed by definite constriction (oviduct) consisting of 12 cells; leads into large elongate spermatotheca filled with spherical sperm in inseminated females. Sperm morphology different from that of sperm in male. Sperm cytoplasm with nucleus located on one side; rest of sperm transparent. Crustaformeria consists of 4 rows of 8 large, rounded cells, each of which forms an elongate tube adjoining spermatotheca by slight narrowing of gonoduct. Crustaformeria followed by another sphincter-like constriction of 8 narrow cells. Uterus proper lined with flat epithelium. Crustaformeria usually longer than spermatotheca or uterus proper in

young females. Up to 12 eggs in uterus at one time in first generation females. Vagina length approximately 1/3 body width; dilation controlled by 8 conspicuous muscles (dilatores vaginae), 4 directed posteriad, 4 anteriad. They appear inserted to body wall between subventral sector of somatic muscles and lateral chord. Four conspicuous nuclei, 2 each situated anteriorly and posteriorly of the vagina in the ventro-median plane belong to the sphincter muscles around the vagina (sphincter vaginae). Each pair of nuclei appears as one large nucleus in lateral view. Females of second generation with shorter gonad and various parts proportionally smaller. Ovary usually outstretched, extends to middle of end bulb, seldom reflexed once. Fewer eggs (3-4) in uterus. Tail conical, narrows in most specimens at approximately 1/6 of its length to peg-like, not sharply pointed, nonannulated tip. Caudalid 3 annules long, 2 to 3 annules anteriad of anus. Phasmids not seen.

MALES:

Measurements—First generation (n = 50): Body length: 1,175.0-1,850.0 μm (1,451.7 μm ± 123.1); body width: 27.5-48.4 μm (38.7 μm ± 4.4); stylet length: 9.6-10.2 μm (10.0 μm ± 0.22); dorsal esophageal gland orifice: 0.8-1.5 μm (1.15 μm ± 0.18); esophagus length: 181.6-287.6 μm (213.3 μm ± 18.6); excretory pore: 125.2-194.6 μm (156.8 μm ± 12.6); anal body diameter: 20.4-30.1 μm (25.2 μm ± 2.0); tail length: 58.7-102.0 μm (84.4 μm ± 7.8); spicule length: 27.5-34.2 μm (30.1 μm ± 1.5); gubernaculum length: 10.2-13.9 μm (11.8 μm ± 1.0); extent of caudal alae: 91.5-137.3 μm (113.1 μm ± 9.2); a: 31.0-47.0 (38.4 ± 4.1); b: 5.8-7.8 (6.8 ± 0.47); c: 14.7-24.4 (17.3 ± 1.6); c′: 2.4-4.2 (3.4 ± 0.34); excretory pore %: 8.1-12.6 (10.8 ± 0.74); T: 66.4-84.6 (75.8 ± 4.9).

Second generation (n = 45): Body length: 1,137.5-1,512.5 μm (1,321.1 μm ± 100.1); body width: 25.7-43.4 μm (33.4 μm ± 4.2); stylet length: 9.2-10.2 μm (9.7 μm ± 0.33); dorsal esophageal gland orifice: 0.8-1.3 μm (1.0 μm ± 0.13); esophagus length: 186.1-242.3 μm (212.0 μm ± 13.9); excretory pore: 137.4-171.8 μm (153.8 μm ± 7.7); anal body diameter: 20.4-27.5 μm (24.6 μm ± 1.7); tail length: 64.1-91.8 μm (77.2 μm ± 6.6); spicule length: 26.5-31.1 μm (29.0 μm ± 1.3); gubernaculum length: 8.8-13.6 μm (10.9 μm ± 1.2); extent of caudal alae: 85.0-123.2 μm (10.1 μm ± 9.6); a: 32.8-48.8 (39.9 ± 3.7); b: 5.2-7.5 (6.3 ± 0.56); c: 13.9-20.2 (17.2 ± 1.3); c′: 2.6-4.0 (3.2 ± 0.36); excretory pore %: 10.1-13.1 (11.7 ± 0.71); T: 60.6-88.5 (72.2 ± 5.8).

Description (Fig. 1, 3)—Smaller than females in both generations; almost straight when heat-killed. Anterior body region (including lip region, stylet, and esophagus) as well as excretory system and intestine similar to those of female. Lateral field narrow, ranges from 3.6-6.0 μm (4.8 μm ± 0.50, n = 60); number of incisures fewer than in females, basically four incisures but increases to seven in some individuals. Deirids more pronounced than in females. Single testis long, may reach basal bulb of esophagus, with distinct cap cell; reflexed once in large individuals of first generation, outstretched in males of second generation. Several rows of spermatogonia in germinal zone; spermatocytes also in multiple or double rows in growth zone. In males of second generation, spermatogonia only for short distance in double rows; spermatocytes in single row. Seminal vesicle long; filled with spherical to ovoid sperm that are densely granular with distinct nuclei. Vas deferens glandular; filled with granular and vacuolate globules of different size. Spicules not fused, curved and massive, with 2 sclerotized thickenings protruding into cytoplasmic core near junction of shaft and blade; blades with sclerotized wings in ventral aspect. Gubernaculum trough-like and curved, sometimes with small distal backward curved process or long fine straight extension. Caudal alae crenate; originate anterior to spicules, posteriorly not enveloping tail tip but stopping short where tip narrows. Tail tip not annulated; ending not sharply pointed. Phasmids not seen.

EGGS:

The eggs are small and broadly cylindrical. They measure 66.8-96.4 μm (76.6 μm ± 6.5) long by 30.6-43.8 μm (36.1 μm ± 3.1) wide (n = 50). The length/breadth ratio is 1.5-3.1 (2.1 ± 0.33). Eggs usually undergo cleavage after

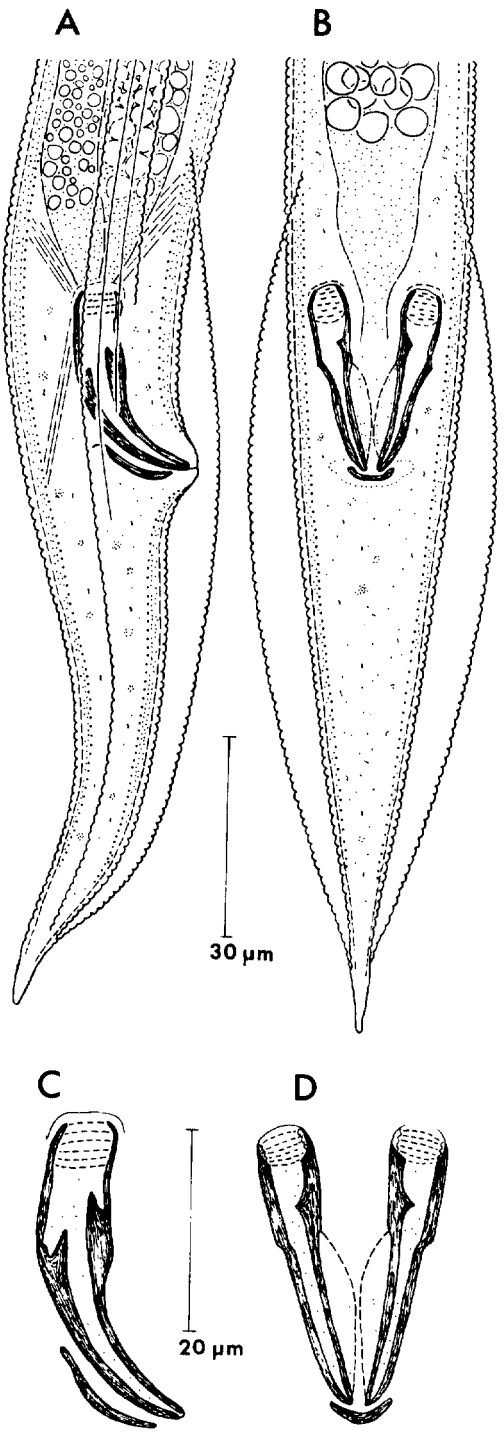

FIG. 3-(A-D). Males of *Anguina plantaginis* n.sp. *A)* Tail (lateral). *B)* Tail (ventral). *C)* Spicule and gubernaculum (lateral). *D)* Spicules and gubernaculum (ventral).

being deposited into the gall cavity. Cleavage may be initiated in the uterus in older females.

INFECTIVE LARVAE (third stage):

Measurements—Female larvae (n = 25): Body length: 630.0-829.8 µm (748.5 µm ± 43.7); body width: 21.9-29.5 µm (25.6 µm ± 2.0); stylet length: 9.2-10.0 µm (9.6 µm ± 0.25); dorsal esophageal gland orifice: 0.9-1.4 µm (1.1 µm ± 0.15); esophagus length: 122.0-151.3 µm (134.9 µm ± 7.9); excretory pore: 93.3-120.6 µm (110.2 µm ± 6.0); gonad length: 20.4-40.8 µm (31.4 µm ± 5.0); gonad end to tail end: 123.3-208.3 µm (172.7 µm ± 19.1); gonad end to anus: 68.4-138.6 µm (110.2 µm ± 17.3); tail length: 54.7-72.4 µm (62.6 µm ± 4.4); a: 26.5-32.6 (29.3 ± 1.5); b: 5.0-6.3 (5.6 ± 0.38); c: 10.9-13.3 (11.9 ± 0.7); excretory pore %: 14.0-15.8 (14.7 ± 0.5); gonad end to tail end %: 17.5-26.4 (23.1 ± 2.4); gonad end to anus %: 9.7-18.2 (14.7 ± 2.3).

Male larvae (n = 25): Body length: 694.8-873.0 µm (785.8 µm ± 46.0); body width: 21.4-28.8 µm (25.8 µm ± 1.9); stylet length: 9.1-9.9 µm (9.4 µm ± 0.21); dorsal esophageal gland orifice: 0.9-1.3 µm (1.1 µm ± 0.14); esophagus length: 124.1-164.9 µm (142.6 µm ± 10.9); excretory pore: 102.5-122.9 µm (112.7 µm ± 5.4); gonad length: 21.6-39.8 µm (28.9 µm ± 4.9); gonad end to tail end: 297.5-425.9 µm (362.5 µm ± 34.9); gonad end to anus: 233.3-357.4 µm (296.5 µm ± 33.5); tail length: 60.0-73.4 µm (66.0 µm ± 3.9); a: 26.6-34.2 (30.5 ± 1.9); b: 4.4-6.4 (5.5 ± 0.41); c: 10.1-13.2 (11.9 ± 0.68); excretory pore %: 13.5-16.8 (14.4 ± 0.72); gonad end to tail end %: 41.2-52.3 (46.2 ± 3.0); gonad end to anus %: 33.2-44.3 (37.8 ± 3.1).

Description (Fig. 5)—Infective larvae move fast in water and have a tendency to swarm. Body straight or slightly curved ventrally when relaxed by gentle heat. Larvae appear dark in transmitted light because of dense intestinal contents and accumulation of reserve material in esophageal region. Cuticle thick, finely annulated. Lateral field distinct, with 4 incisures. Deirids very prominent; located at level of excretory pore. Phasmids not seen. Body musculature appears as clear band

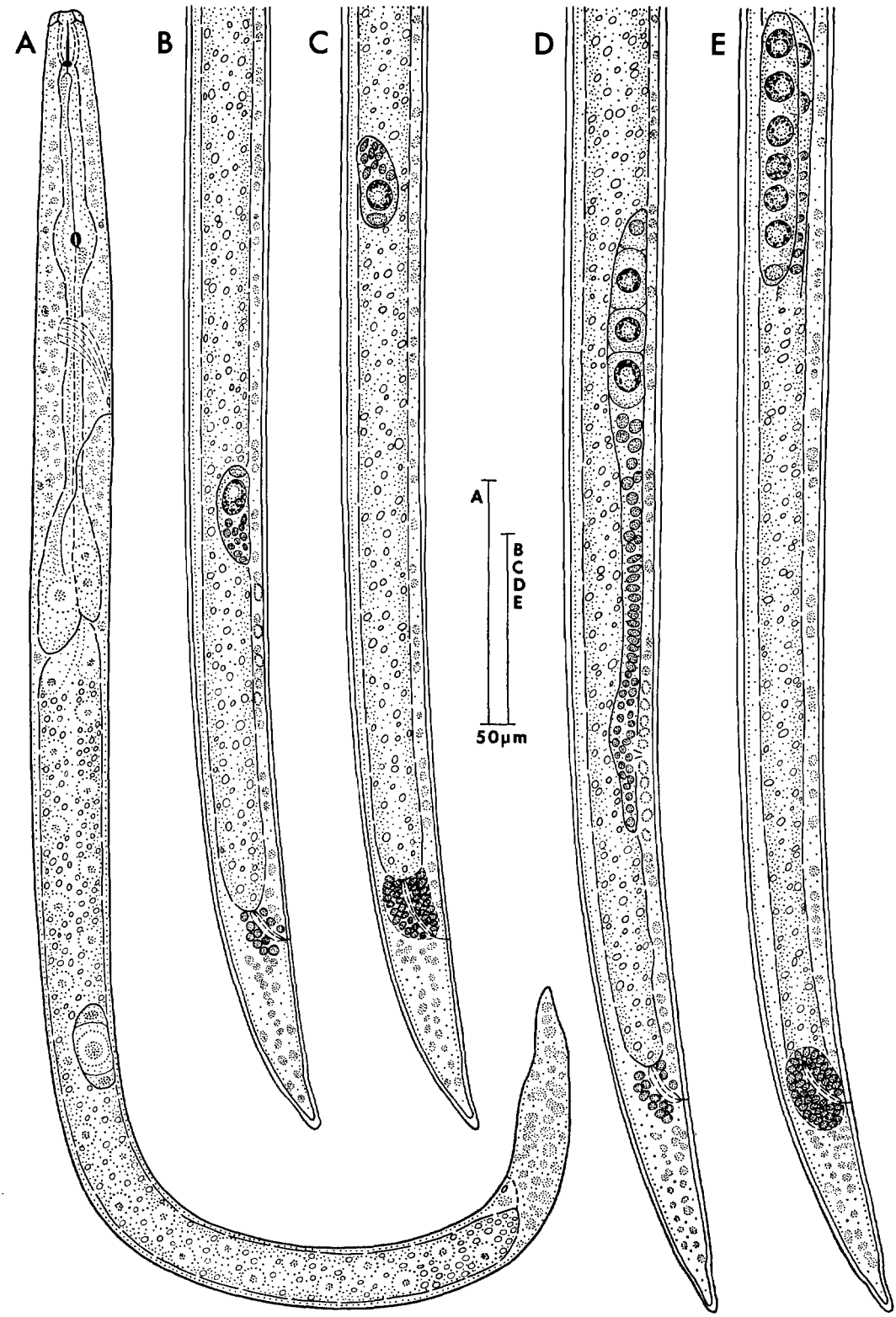

FIG. 4-(A-E). Postembryonic development of *Anguina plantaginis*. *A)* Full-length outline (lateral) of freshly-hatched second-stage larva (specimen curved to fit page size). *B)* Second molt, posterior portion of female larva. *C)* Second molt, posterior portion of male larva. *D)* Third molt, posterior portion of female larva. *E)* Third molt, posterior portion of male larva. B-E stained with orcein.

beneath cuticle. Lip region not set off, smooth and flattened in front, with weakly cuticularized framework. Stylet with small rounded knobs. Dorsal esophageal gland orifice close behind stylet knob base. Esophagus with long narrow isthmus; esophageal glands enclosed in bulbous swelling overlapping intestine dorsally. Dorsal gland nucleus very distinct. Distance of median bulb valve to junction with intestine about 1.5 times the distance of head end to median bulb valve. Nerve ring encircles isthmus near middle. Excretory pore at level of beginning of bulbous swelling. Intestine packed with reserve material; cells not seen. Rectum and anus inconspicuous. Caudalid distinct. Genital primordium with one large central germinal nucleus and several epithelial nuclei. In female larvae, primordium located ventrally with cap cell nucleus anteriad; group of epithelial nuclei posteriad; 4 specialized ventral chord nuclei present. In male larvae, primordium shifted dorsally with cap cell nucleus posteriad and other epithelial nuclei anteriad. Primordium in male larvae near midbody (53.8% of body length); in female larvae primordium much further posteriad (76.9% of body length). Tail conoid with blunt tip.

HOLOTYPE (Female): Isolated from greenhouse culture propagated on *Plantago aristata,* derived from original population collected by J. N. Sasser from type locality in North Carolina, U. S. A. Slide No. T-254t, United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

ALLOTYPE (Male): Same data as holotype. Slide No. T-255t, same collection.

PARATYPES (Females, males, infective larvae, and eggs): Same data as holotype. United States Department of Agriculture Nematode Collection, Beltsville, Maryland (Slide Nos. T-2009p to T-2014p), and University of California Nematode Survey Collection, Davis, California (Slide Nos. UCNC 1580 to 1585).

TYPE HOST AND HABITAT: *Plantago aristata* Michx. Forms hard globular or oval galls on leaves, penduncles, and inflorescences (bracts, sepals, petals).

TYPE LOCALITY: Poole Road, Wake County, north of Raleigh, North Carolina. Road ditch and shoulder of road.

DIAGNOSIS: *Anguina plantaginis* is distinct from other species of *Anguina* by its morphology and its limited parasitism on only one host plant, *Plantago aristata* (14). This new species appears to be most closely related to *A. klebahni* (5), *A. millefolii* (7), *A. mobilis* (1), and *A. moxae* (2) which are also parasitic on dicotyledonous plants.

The females are not coiled watchspringwise as in *A. millefolii, A. klebahni,* and *A. moxae,* but are only slightly curved ventrally in a manner similar to *A. mobilis.*

In body length, *A. plantaginis* resembles *A. millefolii* and *A. mobilis* more and differs from the other species which are either smaller or larger.

The lateral field of *A. plantaginis* has, on the average, fewer incisures than those of *A. mobilis* and *A. moxae,* and the always distinct deirids of *A. plantaginis* have not been observed in the other species on dicotyledonous plants.

A. plantaginis has a smaller stylet than *A. millefolii.* The dorsal gland orifice is situated closely behind the stylet knob base (0.8-1.5 μm), whereas in *A. moxae* it is located further posteriad (2-3 μm).

The first generation adults of *A. plantaginis* have a slightly expanded procorpus and isthmus of the esophagus as they are similarly present in *A. millefolii* and *A. moxae.* The isthmus is, however, not expanded to such an extent that it forms a "storage organ" as it has been described in other *Anguina* species.

The female reproductive system of *A. plantaginis* has one constriction (12 cells = oviduct) between ovary and spermatotheca and another one (8 cells) between crustaformeria and uterus proper. The latter constriction was also described in *A. moxae,* the former in *A. moxae* and *A. millefolii.* The spermatotheca is similar to those of *A. mobilis* and *A. moxae,* which have oblong and ovate to slightly elongated spermatothecae, respectively. The crustaformeria is only slightly longer than the spermatotheca, whereas it is two and a half times the length of spermatotheca in *A. mobilis* and *A. moxae.* The postvulval uterine sac is about one and a half times

the body width at the vulva (45% of vulval-anus distance). *A. plantaginis* resembles *A. klebahni* in this respect and differs from *A. mobilis,* which has a well-developed postvulval uterine sac of almost three times the body width at the vulva (about 70% of vulval-anus distance), and *A. moxae* and *A. millefolii,* which have short, rudimentary, collapsed postvulval sacs. *A. plantaginis* is distinguished from *A. klebahni* by the more posterior vulva position (87% and 85% in first and second generation females, respectively, vs. 70%).

The spicules and gubernaculum of the males of *A. plantaginis* are larger than those of *A. klebahni* and *A. mobilis,* and smaller than those of *A. millefolii.*

The tail tip of both sexes of *A. plantaginis* is not sharply pointed as in the other species. Tails of both sexes, especially those of the males, are long (c = 17.2) in comparison to those of the other species, except for *A. klebahni* and the second generation of *A. millefolii* which have long tails too.

The eggs of *A. plantaginis* differ in size from those of *A. mobilis* which are larger (82-144 μm by 50-47 μm) and from those of *A. klebahni, A. millefolii,* and *A. moxae* which are smaller.

In *A. plantaginis,* as in *A. mobilis,* the third-stage larva is the infective stage; whereas in the other 3 species, it is believed to be the second stage that is infective.

Anguina plantaginis causes galls on leaves, bracts, sepals, and petals of *Plantago aristata* and is thus distinguished from the other species that cause only leaf galls on dicots. An exception to this is *A. klebahni* which infects flower parts of *Primula florindae.*

TABLE 1. Morphometrics of 40 second-stage larvae of *Anguina plantaginis* n. sp.

Character (μm)	Range	Mean	Standard Deviation
Body length	319.5-397.8	361.4	20.6
Stylet length	8.9- 9.3	9.1	0.13
Dors. esoph. gland orifice	0.8- 1.3	1.0	0.14
Gonad length	14.8- 19.6	17.2	1.2
Gonad end to tail end	121.1-165.7	145.9	9.9
Tail length	29.1- 39.0	35.7	2.2
a ratio	19.8- 27.6	23.7	1.9
b ratio	2.5- 3.2	2.9	0.16
c ratio	8.6- 12.8	10.2	0.9
Excretory pore %	20.7- 26.2	22.6	1.2
Gonad end to tail end %	37.1- 44.0	40.4	1.5

POSTEMBRYOGENESIS

The two generations of *Anguina plantaginis* adults that develop in one gall give rise to two generations of larval offspring. First-generation larvae are considerably larger than the corresponding stages of second-generation larvae.

The first molt occurs in the egg, and then the second-stage larva hatches (Fig. 4-A; Table 1). In first-generation larvae, the postembryonic development continues normally through third and fourth stages (Fig. 4, 5; Table 2, 3) and is completed with the second-generation adults. Depending on conditions, however, a variable proportion of the first-generation larvae may develop into infective larvae. Most or all of the second-generation larvae develop into infective larvae.

The measurements and illustrations in the following description of the various larval stages are based on first-generation larvae.

The second-stage larvae are small and very transparent (Fig. 4-A; Table 1). The cuticle is thin and finely annulated. The lateral field has two distinct incisures and the deirids are conspicuous at the level of the excretory pore. Phasmids were not seen. The junction of esophagus and intestine is distinct and the gland lobe overlaps the intestine slightly. The intestine is well outlined; the rectum is indistinct. The genital primordium is large with one big central germinal nucleus and two smaller epithelial nuclei, one on each end. The tail has a blunt tip.

After a period of feeding and growing, the second-stage larvae undergo the second molt (Fig. 4-B, C; Table 2). The epithelial cells of the genital primordium divide during this molt and become characteristically arranged. In female larvae, the primordium grows out posteriorly and four specialized ventral chord nuclei can be distinguished in the ventral chord. In male larvae, the primordium grows out anteriorly and the cells of the spicule

TABLE 2. Morphometrics of second- and third-molt females and males of *Anguina plantaginis* n. sp.

Character (μm)	Females			Males		
	Range	Mean	Standard Deviation	Range	Mean	Standard Deviation
Second molt (n = 25)						
Body length	465.3-612.0	538.6	35.0	459.0-601.2	546.1	44.4
Gonad length	22.4- 38.9	29.3	4.1	18.4- 30.6	24.5	3.2
Gonad end to tail end	144.5-263.1	199.8	33.2	189.6-266.5	232.4	23.2
Tail length	40.3- 56.4	49.1	4.2	43.9- 54.9	49.0	3.4
a ratio	21.3- 26.5	24.1	1.3	21.6- 27.6	24.5	1.4
b ratio	3.7- 5.0	4.2	0.31	3.6- 4.9	4.2	0.36
c ratio	9.5- 12.4	11.0	0.7	10.1- 13.2	11.2	0.8
Excretory pore %	14.4- 18.8	16.8	1.0	14.7- 19.5	17.0	1.1
Gonad end to tail end %	26.8- 46.1	37.1	5.4	36.0- 46.8	42.6	2.5
Third molt (n = 20)						
Body length	679.5-829.8	765.0	46.5	678.6-825.3	759.4	44.9
Gonad length	79.9-163.2	125.3	21.7	64.3-155.6	115.2	27.2
Gonad end to tail end	102.0-174.3	136.1	18.2	230.4-349.8	300.0	37.7
Tail length	51.0- 66.3	59.8	4.4	53.0- 65.3	59.6	4.1
a ratio	26.8- 31.9	29.4	1.4	25.4- 33.0	29.3	1.9
b ratio	4.1- 5.0	4.7	0.31	3.9- 5.8	4.7	0.53
c ratio	11.7- 4.3	12.8	0.7	11.6- 14.1	12.8	0.7
Excretory pore %	12.8- 15.8	14.7	0.9	13.5- 16.2	14.4	0.6
Gonad end to tail end %	13.9- 22.0	17.8	2.1	32.0- 44.2	39.4	3.3

primordia are evident in the rectal area. Whereas the posterior end of the genital primordium of the male remains located, on the average, at 57.4% of the body length, that of the female is located further posteriorly, at a point about 63% of the body length. Thus, the sexes can be distinguished as early as the second molt.

The third-stage larvae continue to grow in length, and the differences between the sexes become more pronounced as the genital primordium gradually lengthens. In female larvae, the epithelial cells that later will form the gonoduct continue to grow posteriorly; in males, these cells also multiply and the primordium extends anteriorly.

During the third molt (Fig. 4-D, E; Table 2), the germinal nuclei begin to divide. The genital primordium of male larvae increases in length and its anterior end turns and elongates posteriorly. The developing gonad thus attains an inverted U-shape. Later in the molt, it straightens and the earlier posteriorly located part, including up to eight germinal nuclei, extends anteriorly. The posterior gonad tip is located at 60.6% of the body length. The spicule primordia increase in size. In females, the gonad also increases in length and extends further posteriad to include up to 10 germinal nuclei anteriorly and 16 specialized ventral chord nuclei posteriorly near the developing vaginal primordium. The posterior gonad tip moves considerably posteriad and lies on the average at 82.2% of the body length.

The infective larvae, which often were observed at this time, are of the same size as the fully grown, third-stage larvae at the onset of the third molt (compare measurements of infective larvae, p. 235 with those of third-molt larvae in Table 2). Such infective larvae, however, are morphologically different from the normally developing third-stage larvae (compare Fig. 4-D, E with Fig. 5-C, D). The esophagus is shorter than that of the advanced third-stage larvae. The gonad is of the same size as that of second-molt or early third-stage larvae and is located further anteriad than in the third-molt larvae. The body contents appear very dense because of the presence of storage materials that obscure the internal organs.

The dimensions of fully grown fourth-stage larvae are listed in Table 3. During the fourth stage, the gonads continue to

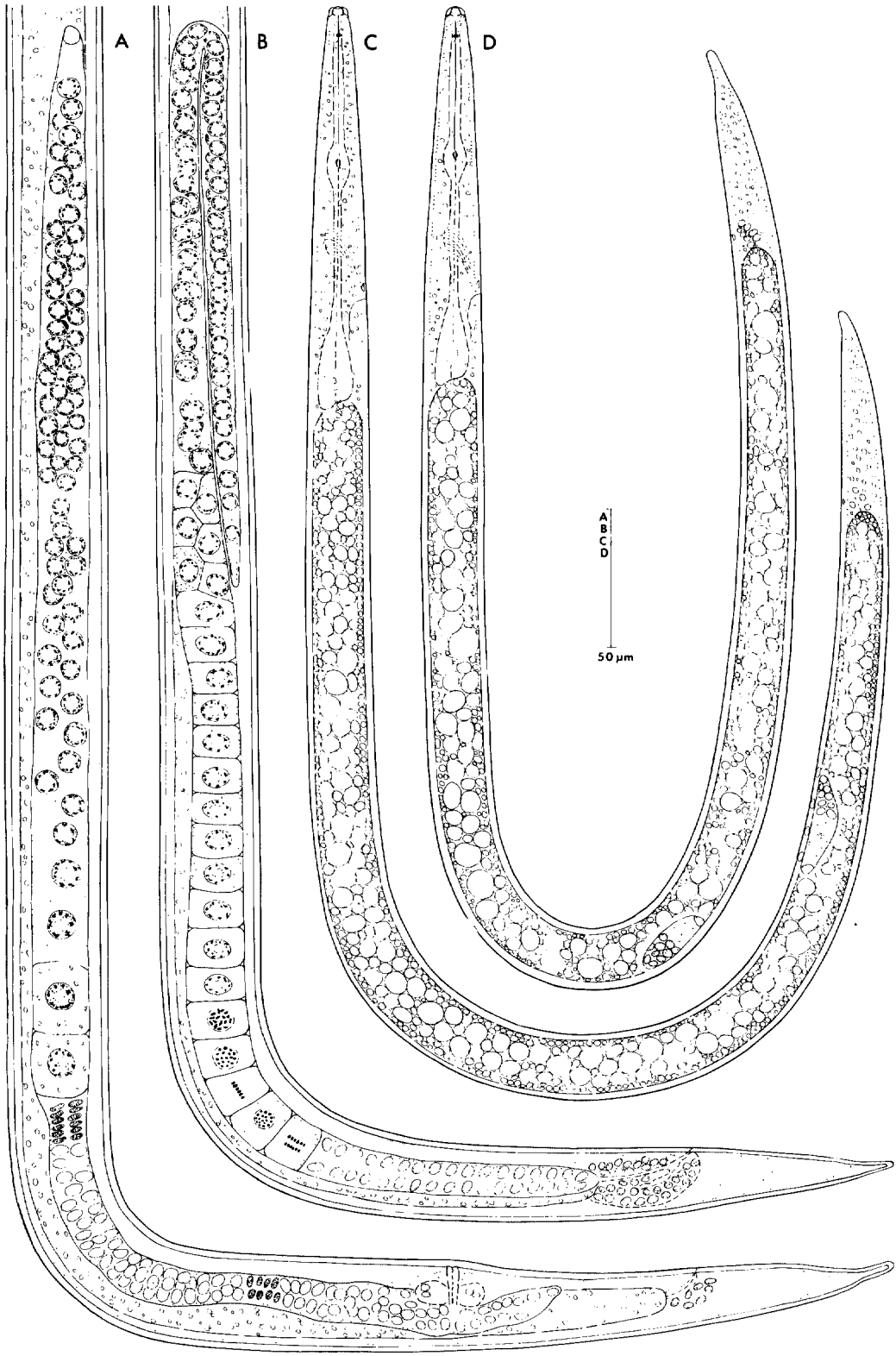

FIG. 5-(A-D). Postembryonic development of *Anguina plantaginis*. *A)* Fourth molt, posterior portion of female larva. *B)* Fourth molt, posterior portion of male larva. *C)* Full-length outline of female infective larva. *D)* Full-length outline of male infective larva. A, B stained with orcein. All specimens curved for convenience in illustration.

elongate and begin to differentiate into the various parts. In the female, the postvulval uterine sac begins to differentiate as the rudiment of the vagina becomes visible. The specialized ventral chord nuclei continue to migrate inside and clearly indicate the position of the future vagina. The number of oogonial nuclei increases to 28. In the male, the developing gonads grow both anteriorly and posteriorly, and the elongating vas deferens connects with the enlarged spicule primordia. There may be up to 58 spermatogonial nuclei in males approaching the fourth molt.

When infective larvae are kept in water for 2-3 weeks, they begin to molt into fourth-stage larvae. A new cuticle and stylet are formed during this molt, and the small genital primordium rapidly grows out to the full-size gonad of a normally developing fourth-stage larva. During this molt, therefore, the genital primordium undergoes all the changes that regularly occur during the third stage and third molt of the normally developing larva.

During the fourth molt, differentiation of the various gonad parts is completed (Fig. 5-A, B; Table 3). In females, the ovary may contain up to 75 oogonia and oocytes, and vulva and vagina formation are completed. In males, spicules, gubernaculum, and caudal alae are formed and the number of spermatogonia and spermatocytes increases to around 90. Already at the beginning of the fourth molt, maturation divisions occur, and around 20 spermatids may be present in the seminal vesicle.

DISCUSSION

Two generations of *A. plantaginis* often occurred within galls, especially larger ones of 3-5 mm diam, and first-generation adults were larger in size than those of the second generation. The same has been observed also in *A. millefolii* (7) and *A. mobilis* (1). When few (2 to 4) nematodes were present in a gall formed by *A. agropyronifloris* Norton, they were larger; whereas when many (over 40) were present, the individuals were smaller in size (10). This

TABLE 3. Morphometrics of fourth-stage and fourth-molt females and males of *Anguina plantaginis* n. sp.

Character (μm)	Females			Males		
	Range	Mean	Standard Deviation	Range	Mean	Standard Deviation
Fourth stage (n = 20)						
Body length	952.5-1,110.0	1,006.1	46.2	912.5-1,107.5	993.6	57.6
Stylet length	9.2- 10.0	9.6	0.23	9.2- 10.0	9.5	0.28
Dors. esoph. gland orifice	1.0- 1.4	1.2	0.13	0.9- 1.4	1.2	0.17
Gonad length	245.7- 340.0	285.8	26.6	212.5- 440.3	305.0	63.0
Gonad end to tail end	157.3- 212.5	176.4	14.5	123.3- 283.9	219.1	38.0
Tail length	62.0- 77.1	68.4	3.9	61.2- 81.6	69.8	5.3
a ratio	36.6- 44.2	40.5	2.2	37.5- 44.2	40.3	2.0
b ratio	4.5- 5.5	5.0	0.28	3.4- 5.4	4.7	0.45
c ratio	12.7- 16.5	14.8	1.1	12.6- 15.8	14.3	0.9
Excretory pore %	12.6- 14.4	13.2	0.5	12.4- 14.4	13.6	0.5
Gonad end to tail end %	16.1- 19.9	17.5	1.2	12.8- 30.7	22.1	3.8
Fourth molt (n = 10)						
Body length	905.0-1,162.5	1,061.8	93.1	1,025.0-1,145.0	1,080.7	41.0
Gonad length	412.3- 672.8	565.1	86.2	603.5- 835.6	732.2	75.4
Postvulval uterine sac	29.8- 63.8	42.5	10.3			
Tail length	66.7- 75.0	71.0	3.3	69.4- 79.0	73.4	3.1
a ratio	27.9- 32.9	29.8	1.9	26.8- 33.1	29.7	2.1
b ratio	5.6- 6.9	6.1	0.55	5.4- 7.4	6.2	0.59
c ratio	13.4- 16.3	15.0	0.9	13.9- 16.5	14.8	1.0
Excretory pore %	11.5- 13.6	12.3	0.7	10.4- 14.7	12.1	1.1
Gonad length %	45.6- 60.1	53.1	5.1	52.7- 80.3	67.9	8.2
Postvulval uterine sac %	2.7- 5.6	3.9	0.88			

observation may also indicate that two generations occur within one gall: the few large adults represent the first generation and the many smaller adults, the second generation. In contrast, only one generation of adults was found in galls formed by *A. tritici* and most other *Anguina* species.

It appears that the differences in body size between the first and second generation are determined by the amount of food and space available to each individual. As a result of crowding, adults of the second generation become smaller. The considerable difference in size between first- and second-generation adults indicates that certain body measurements in species of this genus are of limited value as differentiating characters.

The postembryogenesis of *A. plantaginis,* including the development and differentiation of the reproductive system, is basically similar to that of *Ditylenchus triformis* Hirschmann and Sasser (8). Sex can be distinguished from the second molt on by morphological differences and location of the developing genital primordium. The number of germinal as well as epithelial nuclei is considerably larger in more advanced stages of both sexes of *A. plantaginis,* in comparison to the number present in advanced stages of *D. triformis.* Cell divisions in the reproductive system apparently occur throughout molts and larval stages.

In general, it has been stated in the literature that in *Anguina,* the first-stage larva hatches from the egg and the second-stage larva is the infective stage (3). This stage undergoes dormancy and is resistant to adverse environmental conditions. Exceptions to this are *A. calamagrostis* Wu, *A. balsamophila,* and *A. graminophila* (T. Goodey) in which the fourth stage has been reported as infective (6, 12, 15). In *A. microlaenae* (Fawcett), the first-stage larvae have been described as infective (4). In *Nothanguina cecidoplastes* (T. Goodey) and *A. mobilis,* the third stage has been found to be the infective stage which undergoes two more molts in the host tissue before becoming adult (1, 9). Third-stage larvae appear to be the infective stage also in *A. plantaginis.* As I confirmed in this species, the first molt occurs within the egg, and it is the second-stage larva that hatches. The development proceeds through third and fourth larval stages and, during the latter part of postembryogenesis, some infective larvae can be distinguished by their small genital primordium and the accumulation of reserve material in esophagus and intestine. Although such infective larvae were first detected late in postembryogenesis, it is suspected that infective larvae had started differentiating much earlier. However, the fact that infective larvae molted into normal fourth-stage larvae when they were kept in water confirms that infective larvae are truly third-stage larvae. Upon closer examination, it may be found that infective larvae of all *Anguina* species may be third-stage larvae. If this proves to be true, the infective stage of the plant parasites in the genus *Anguina* will be comparable to the infective stage of animal parasites and the dispersal stage of free-living forms which also undergo cryptobiosis in the third larval stage.

LITERATURE CITED

1. CHIT, W., and J. M. FISHER. 1975. Anguina mobilis n. sp., a parasite of cape weed (Arctotheca calendula). Nematologica 21:53-61.
2. CHOI, Y. E., and P. A. A. LOOF. 1973. Redescription of Anguina moxae Yokoo and Choi, 1968. Nematologica 19:285-292.
3. COOPER, A. F., JR., and S. D. VAN GUNDY. 1971. Senescence, quiescence and cryptobiosis. Pages 297-318 *in* B. M. Zuckerman, W. F. Mai and R. A. Rohde, eds. Plant parasitic nematodes. Vol. 2. Academic Press, New York, N. Y.
4. FAWCETT, S. G. M. 1938. A disease of the Australian grass Microlaena stipoides R. Br. caused by a nematode, Anguillulina microlaenae n. sp. J. Helminthol. 16:17-32.
5. GOFFART, H. 1942. Anguina klebahni n. sp. (Tylenchidae), ein Nematode in Blüten von Primula florindae Ward. Zool. Anz. 138:174-179.
6. GOODEY, T. 1933. Anguillulina graminophila n. sp., a nematode causing galls on the leaves of fine bent-grass. J. Helminthol. 11:45-56.
7. GOODEY, T. 1938. Observations on Anguillulina millefolii (Löw, 1874) Goodey, 1932, from galls on the leaves of yarrow, Achillea millefolium L. J. Helminthol. 16:93-108.
8. HIRSCHMANN, H. 1962. The life cycle of Ditylenchus triformis (Nematoda:Tylenchida) with emphasis on postembryonic development. Proc. Helminthol. Soc. Wash. 29:30-43.
9. KUMAR, A. C. 1969. A note on the occurrence of fourth stage larvae in the life cycle of Nothanguina cecidoplastes. Nematologica 15: 165-167.

10. NORTON, D. C. 1965. Anguina agropyronifloris n. sp., infecting florets of Agropyron smithii. Proc. Helminthol. Soc. Wash. 32:118-122.
11. SEINHORST, J. W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica 4:67-69.
12. THORNE, G. 1926. Tylenchus balsamophilus, a new plant parasitic nematode. J. Parasitol. 12:141-145.
13. TRIANTAPHYLLOU, A. C., and H. HIRSCHMANN. 1966. Gametogenesis and reproduction in the wheat nematode, Anguina tritici. Nematologica 12:437-442.
14. VARGAS, O. F., and J. N. SASSER. 1976. Biology of Anguina plantaginis parasitic on Plantago aristata. J. Nematol. 8:64-68.
15. WU, L.-Y. 1967. Anguina calamagrostis, a new species from grass, with an emendation of the generic characters for the genera Anguina Scopoli, 1777 and Ditylenchus Filipjev, 1936 (Tylenchidae: Nematoda). Can. J. Zool. 45: 1003-1010.