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PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

VOLUME 25 JULY, 1958 NUMBER 2

Three New Species of the Genus *Paratylenchus* from Canada (Nematoda: Criconematidae)*

GEORGIANNA L. BROWN**

Species of *Paratylenchus* with very long, flexible spears were collected on several occasions. Three of these are described herein. Two of the species, *P. aciculus,* n. sp., and *P. aculentus,* n. sp., are so small that they are easily overlooked in soil screenings. These are ectoparasites of grasses and were collected from low, damp areas. *P. audriellus,* n. sp., is apparently an ectoparasite of native trees. These three species are of particular interest because of their close relation to the genus *Cacopaurus* Thorne, 1943. The larvae of *P. aciculus* and *P. audriellus* both have well-developed spears, which Thorne (1943) considered to be characteristic of the genus *Cacopaurus* and not of *Paratylenchus.* No larvae of *P. aculentus* was collected. The females of *P. aciculus* and *P. aculentus* do not have lateral membranes over the vulvar opening, contrary to Thorne and Allen (1950), who suggested that the presence of these membranes may be a character of the genus *Paratylenchus.* However, *P. aciculus, P. aculentus,* and *P. audriellus* all show the general generic characters of *Paratylenchus* as outlined by Micoletzky, 1922.

Paratylenchus aciculus, n. sp. (Figs. 1, 2)

DIMENSIONS: 25 females: L = .278 mm. (.24-.31); a = 21.3 (18.4-23.6); b = 2.6 (2.4-2.7); c = 12.4 (10-15.9); V = 70% (68.3-73.5); stylet = 67 μ (61-69).

3 males: L = .284 mm. (.261-.307); a = 25.8 (23.7-27.9); c = 11 (10.9-11.3); spicule = 15.5 μ (14.5-16.5).

FEMALE: Very small, slender. Body tapering uniformly from above vulva to a finely rounded tail tip. Cuticle marked by distinct transverse striae, interrupted in the lateral fields by 3 incisures that appear as 2 bright lines with a fainter one between. Lip region continuous with body contour, with distinct, rounded lips. When head observed from a face view (Fig. 1, C), 4 small, round lips appearing slightly more elevated than the two broader lateral lips. Labial papillae appearing as 4 minute dots.

Spear flexible, very long and slender (Fig. 2). Spear slightly curved when specimens relaxed by gentle heat. Small muscles visible around basal spear knobs in live specimens. Dorsal esophageal gland opening into esophageal lumen about 5 μ behind spear knobs. Conspicuous excretory pore on ventral side in region of median esophageal bulb. Esophagus consisting of a long, narrow precorpus which widens into the valvulated median bulb and a small posterior esophageal bulb distinctly separated from intestine. Cells throughout the intestine not uniformly filled with granules, giving vacuolated effect.

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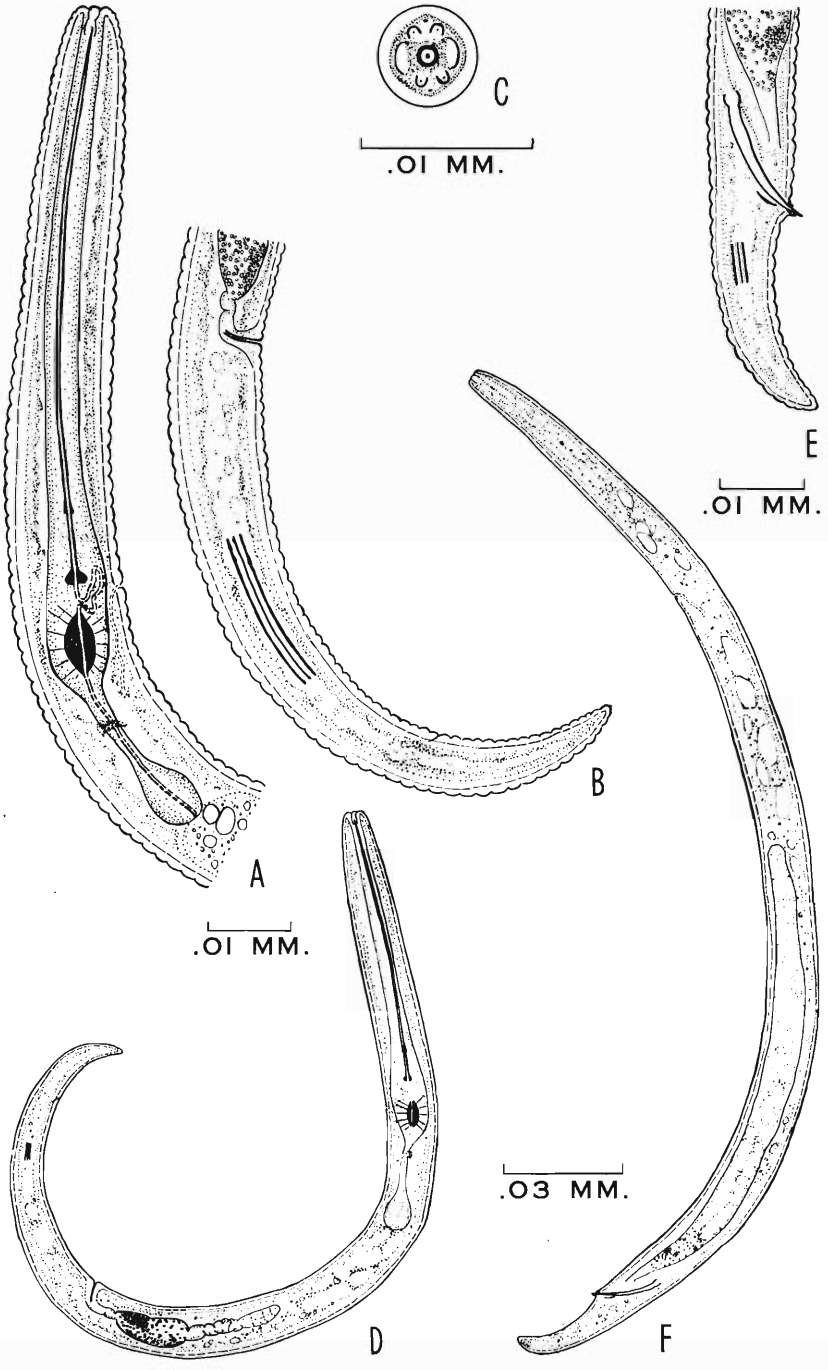

Fig. 1. *P. aciculus*: A, female neck region; B, female tail; C, female face view; D, female, full length; E, male tail; F, male, full length.

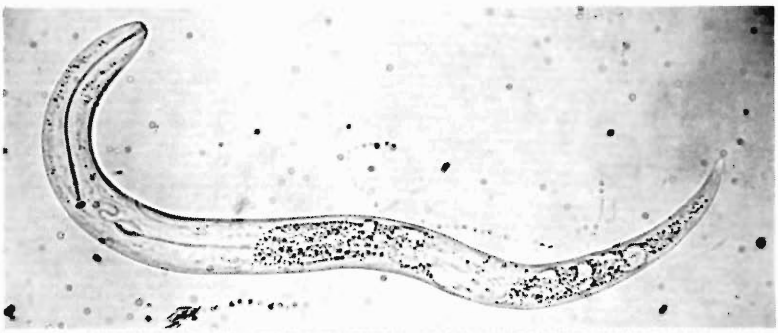

Fig. 2. Photograph of a live specimen of *P. aciculus* showing long, flexible spear and inconspicuous vulva. X400.

Vulva inconspicuous, without lateral membrane. Vagina extending directly inward less than half the diameter of the body. Numerous small sperm in uterus. Ovary outstretched. No postuterine sac. Obscure anus visible in live specimens.

MALE: Males rare. Body about the same size as that of female, with similarly shaped lips. Lateral field marked by 3 incisures. Stylet and esophagus lacking. Large vacuoles in body cavity. The ventrally curved spicula surrounded by an anal sheath, which has a short dorsal extension. Gubernaculum simple. Tail slightly concave, narrowing to a finely rounded tip.

LARVA: Larva with a well-developed spear, 34 μ long, and distinct esophagus. Body forming a loose coil when in normal relaxed position.

TYPE SPECIMENS: Holotype, female, collected in September, 1955, Collection No. 261; allotype, male, same data as holotype; 46 paratypes; all in Canadian Collection of Nematodes.

TYPE HABITAT: Soil about roots of fowl blue-grass (*Poa palustris* L.).*

TYPE LOCALITY: Three miles south of Blackburn, Ontario.

Distribution: This species was also collected from meadow sod near Nesbitt, Manitoba.

DIAGNOSIS: The females of *P. aciculus* differ from all other species of *Paratylenchus* in having a longer spear (67 μ). Other species with spears nearly comparable in size are *P. aculentus* n. sp. (58 μ), *P. goodeyi* Oostenbrink, 1953 (51 μ), *P. audriellus* n. sp. (51 μ), and *P. anceps* Cobb, 1923 (45 μ). The spear length of *P. anceps* was misinterpreted as 67 μ in Oostenbrink (1953).

The presence of 3 lateral lines and the anterior position of the vulva distinguish *P. aciculus* from all others of the genus except *P. aculentus* n. sp., from which it differs in having conspicuous lips and in having a more tapering tail.

Males of *P. aciculus* do not have a spear.

Paratylenchus aculentus n. sp. (Fig. 3)

DIMENSIONS: 10 females: L = .275 mm. (.264-290); a = 20.9 (20-22); b = 2.6 (2.6-2.7); c = 11.3 (10-12); V = 71.6% (70.7-73); stylet = 58.1 μ (54-62).

1 male: L = .308 mm; a = 23.6; c = 11.8; spicule = 15 μ.

* Identified by Dr. W. G. Dore, Botany and Plant Pathology Division, Department of Agriculture, Ottawa, Canada.

FEMALE: Very small, slender. Anteriorly the body tapering a little to a rounded lip region. Posteriorly the body tapering gradually from above vulva to a rounded tail tip. Cuticle marked by distinct transverse striae. In lateral field 3 incisures appearing as 2 bright lines with a fainter line between. When head observed from a face view (Fig. 3, C), 6 obscure lobes visible.

Spear very long and fine, with small, rounded basal knobs. Orifice of dorsal esophageal gland about 7 μ behind spear knobs. Precorpus of esophagus narrow, gradually broadening into the median bulb, which has a strong valvular apparatus. Isthmus of esophagus narrow, surrounded by nerve ring. The posterior esophageal bulb varying somewhat in shape with the expulsion of the spear. Conspicuous excretory pore on ventral side in region of median esophageal bulb. Deirids observed in live specimens, laterad to median bulb. Intestine vacuolate in appearance, with transverse bands of fat globules.

Vulva inconspicuous, without lateral membrance. Vagina extending directly inward about half the diameter of the body. No postuterine sac. Numerous small sperm in broad uterus. The single, outstretched ovary comparatively short. Anus obscure.

MALE: Males rare. Body uniformly cylindrical, tapering to a rounded tail tip similar to that of female. Lateral field marked by 3 incisures. Stylet and esophagus lacking. Intestine vacuolate in appearance. Spicule sharply pointed. Gubernaculum simple. No anal sheath visible on single male collected.

TYPE SPECIMENS: Holotype, female, collected in August, 1955, by R. C. Russell. Collection No. 392A; allotype, male, same data as holotype; 18 paratypes; all in Canadian National Collection of Nematodes.

TYPE HABITAT: Soil about roots of grass sod.

TYPE LOCALITY: Lake Lenore, Saskatchewan.

DISTRIBUTION: Collections of this species also taken from soil about the roots of grama grass (*Bouteloua gracilis* Lag.)* near Manyberries, Alberta; from the roots of grass sod composed of *Calamagrostis canadensis* (Michx.) Beauv.* and *Muhlenbergia mexicana* (L.) Trin.* in Gatineau Park, Quebec.

DIAGNOSIS: *P. aculentis* is most closely related to *P. aciculus,* from which it differs in the shorter spear of the female, in the lack of conspicuous lips, and in having a broader tail in both male and female. *P. aculentus* differs from *P. goodeyi* in the more anterior position of the vulva, in having three lateral lines, and in lacking a spear in the male.

Paratylenchus audriellus n. sp. (Fig. 4)

DIMENSIONS: 15 females: L = .335 mm. (.304-.381); a = 20.2 (17.6-22.9); b = 3.6 (3.1-4.4); c = 14.1 (11.0-18.9); V = 81.6% (79.4-83); stylet = 51.2 μ (48-55).

15 males: L = .328 mm. (.307-.360); a = 27.3 (25.1-30); c = 11.4 (10-12.8); spicule = 19 μ (17.5-22.5).

FEMALE: Cuticle marked by distinct transverse striae about 1 μ apart. The lateral field occupying one-quarter the body width and is marked by 4 bright lines that begin about 25 μ from the head and continue to the tail tip. The body tapering in the esophageal region, ending without constriction in a narrow, truncate head. The small lip region with 6 obscure lobes, which are visible only in face view (Fig. 4, C). Labial papillae appearing as min-

*Identified by Dr. W. G. Dore, Botany and Plant Pathology Division, Department of Agriculture, Ottawa, Canada.

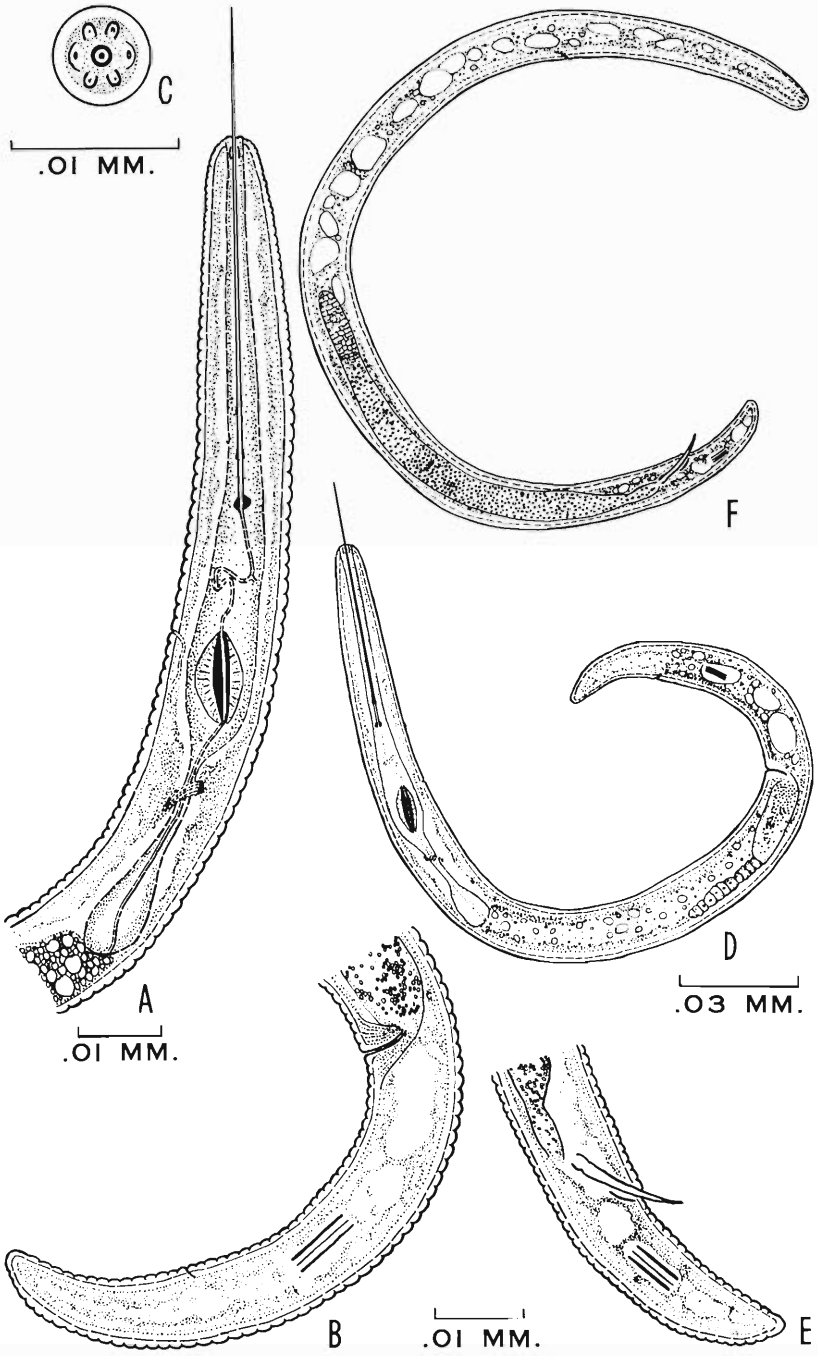

Fig. 3. *P. acutentus*: A, female neck region; B, female tail; C, female face view; D, female, full length; E, male tail; F, male, full length.

ute dots at the apices of the four smaller lobes. Tail tapering from behind vulva to a characteristic clawlike process at tip (Fig. 4, B), this process varying slightly in width between specimens but always ending in a sharp point.

Spear very long and slender, occasionally curved in dead specimens. Spear knobs rounded. Conspicuous excretory pore opening ventrally opposite isthmus of esophagus. Precorpus of esophagus narrow, gradually expanding into the median bulb, which contains the usual refractive valvular apparatus. Pyriform basal esophageal bulb distinctly separated from intestine. Conspicuous groups of variable-sized granules in intestine. Anus obscure.

Vulva a broad transverse slit with conspicuous lateral membranes. Ovary outstretched, overlapping esophagus in some specimens. Uterus large, anteriorly ending in a round spermatheca filled with comparatively large sperm. Small postvulvar rudiment of uterus present.

MALE: Males common. Body slenderer than that of female, forming a slight curve in normal relaxed position (Fig. 4, F). Stylet and esophagus lacking. Lower part of testis filled with comparatively large sperm. Ventrally curved spicula surrounded by a sheath with a hooklike process on the posterior margin. The conoid-arcuate tail ending with a clawlike process similar to that of the female.

LARVA: Larva with well-developed stylet, 47.6 μ. Esophagus distinct. Tail tip with characteristic process not fully formed.

TYPE SPECIMENS: Holotype, female, collected August, 1957, by Audrey James, Collection No. 997; allotype, male, same data as holotype; 40 paratypes; all in Canadian National Collection of Nematodes.

TYPE HABITAT: Soil about the roots of white birch (*Betula papyrifera* Marsh.).

TYPE LOCALITY: Two miles south of Orleans, Ontario.

DISTRIBUTION: This species was also collected about the roots of beech (*Fagus grandifolia* Ehrh.) on Isle d'Orleans, Quebec; quaking aspen (*Populus tremuloides* Michx.) near Dwyer Hill, Ontario; and pin cherry (*Prunus pensylvanica* L.) near Haliburton, Ontario.

DIAGNOSIS: The presence of a distinct clawlike process on the tail of both male and female distinguishes *P. audriellus* from other species of *Paratylenchus*. The long, flexible spear distinguishes it from most species of the genus. *P. audriellus* is closely related to *P. goodeyi*, from which it differs in the male not having a spear.

SUMMARY

Paratylenchus aciculus, n. sp., and *P. aculentus*, n. sp., are ectoparasites of grasses, and *P. audriellus*, n. sp., is an ectoparasite of trees. These are described and figured. Females of all three species have very long flexible spears, while spears are absent in the males. *P. aciculus* and *P. aculentus* are small forms and differ from other species in the genus by having three lateral lines and with a more anterior position of the vulva. *P. aciculus* has a longer spear and more conspicuous lips than *P. aculentus*. *P. audriellus* is distinctive by having a clawlike process at the end of the tail of both male and female. The close relationship of these three new species of *Paratylenchus* with the genus *Cacopaurus* Thorne, 1943, is pointed out.

LITERATURE CITED

COBB, N. A. 1923. Notes on *Paratylenchus*, a genus of nemas. Contrib. Sci-Nematology 14: 367-370.

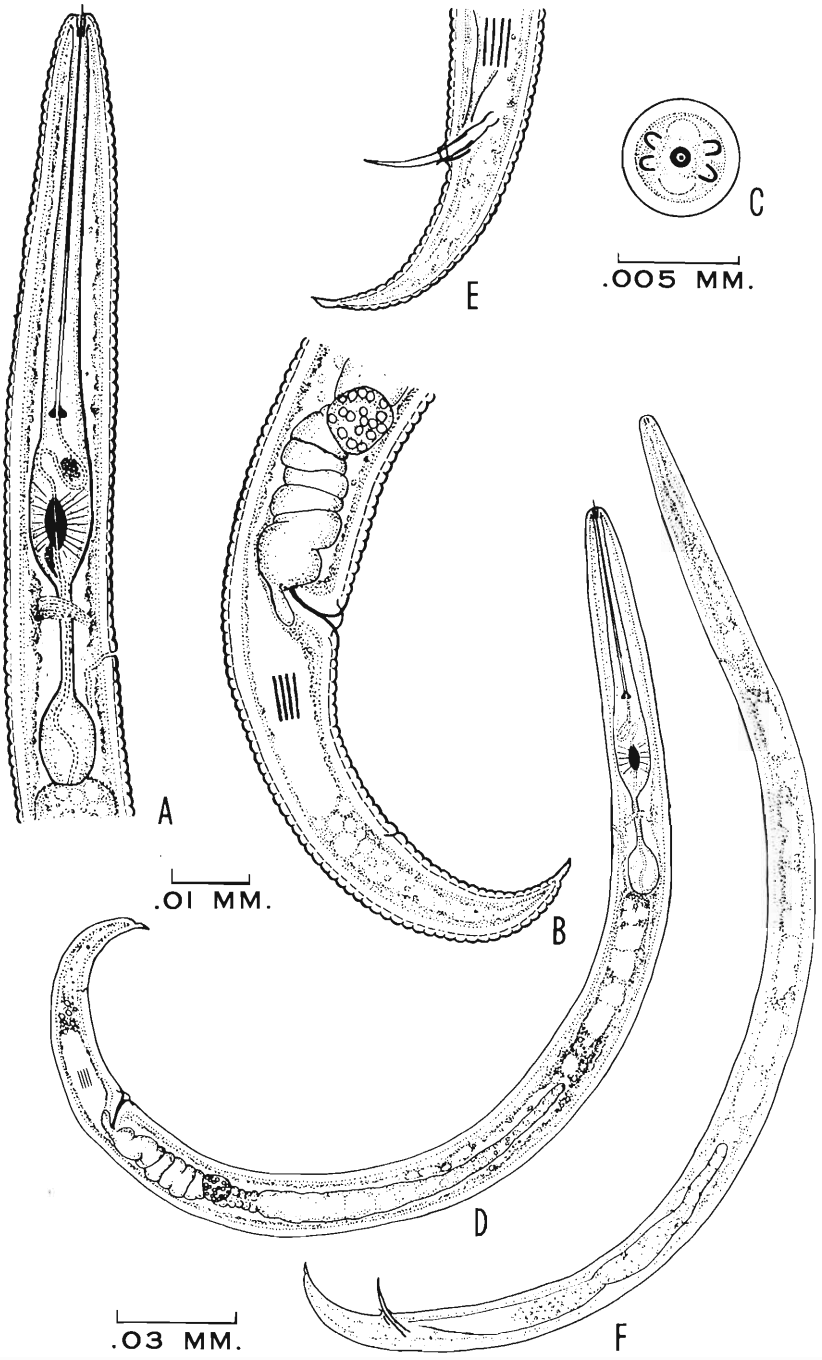

Fig. 4. *P. audriellus*: A, female neck region; B, female tail; C, female face view; D, female, full length; E, male tail; F, male, full length.

MICOLETZKY, H. 1922. Die freilebenden Erdnematoden. Arch. Naturg., A, 87: 605-607.

OOSTENBRINK, M. 1953. A note on *Paratylenchus* in the Netherlands with the description of *P. goodeyi* n. sp. (Nematoda, Criconematidae). Tijdschr. PlZiekt. 59: 207-216.

THORNE, G. 1943. *Cacopaurus pestis*, nov. gen., nov. spec. (Nematoda: Criconematinae), a destructive parasite of the walnut *Juglans regia* Linn. Proc. Helminth. Soc. Wash. 10: 78-82.

THORNE, G., and ALLEN, M. W. 1950. *Paratylenchus hamatus* n. sp. and *Xiphnema index* n. sp., two nematodes associated with fig roots, with a note on *Paratylenchus anceps* Cobb. Proc. Helminth. Soc. Wash. 17: 27-35.

Action of Piperazine Against Mixed Infections of *Ancylostoma caninum* and *Uncinaria stenocephala* in Dogs

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The marked efficiency of piperazine against large roundworms in dogs and other animals has been recognized for several years, but the chemical has not been uniformly effective against hookworms. Guilhon (1951) reported that no *Ancylostoma caninum* eggs were found in the feces of dogs after treatment with pure diethylene diamine (piperazine) in dosages ranging from 60 to 200 mg. per kilogram of body weight. On the other hand, Sloan *et al.* (1954) found that piperazine was rather ineffective against *A. caninum* but very effective against *Uncinaria stenocephala*. In critical trials, all of 38 *Uncinaria* were eliminated from 2 dogs that were given piperazine adipate at 100 mg. per kilogram of body weight; and in 5 additional dogs, post-treatment fecal examinations showed that the eggs of this species were eliminated completely or markedly reduced. Comparable results were not obtained against *Ancylostoma* in 16 dogs that were given dosages ranging from 100 to 300 mg. per kilogram of body weight for 3 consecutive days. As shown by fecal examination after treatment, none of these animals became negative, and post-mortem examination of 4 of the dogs revealed that the chemical had removed only 27 percent (22) of 81 worms. The unreliable action of piperazine against *Ancylostoma* has been noted also by Mann *et al.* (1955), Kotarba *et al.* (1956), Bradley *et al.* (1956), and others.

Because of the wide use of piperazines in small-animal therapeutics, it seemed desirable to obtain additional critical data on the action of the chemical in dogs and, particularly, to evaluate the net usefulness of the chemical against *A. caninum* and *U. stenocephala*.

PROCEDURE

Experimental infections of *U. stenocephala** were established in 16 dogs of mixed breeds ranging from 3 weeks to 1 year of age. The animals were subsequently confined in runs known to be contaminated with infective stages of *A. caninum* in order to obtain concomitant infections with this species. After patency was established, critical trials were undertaken with the test

*The larvae were obtained through the courtesy of Dr. T. W. M. Cameron, MacDonald College, Quebec, Canada.

animals in accordance with the procedure described by Colglazier and Enzie (1951). One or 2 days before treatment, the animals were isolated in individual cages and the feces screened in order to detect any natural elimination of parasites. The chemicals (piperazine citrate and piperazine sulfate) were given in hard gelatin capsules, usually after a fast of 18 to 26 hours, or in a small amount of canned dog feed. In most instances, the animals were returned to regular feed in 2 to 3 hours, but a few were fed immediately after medication. All dosages were calculated in terms of piperazine base. The feces of each animal were collected daily and screened for parasites in the usual manner. When the elimination of parasites ceased, usually within 3 or 4 days, the animals were necropsied and the gastrointestinal tract was examined for parasites and lesions. Three dogs were each given a tranquilizer pill** an hour before treatment in an attempt to prevent vomition.

OBSERVATIONS AND DISCUSSION

The data are given in table 1. At dose rates ranging from 25 to 75 mg. per pound of body weight, piperazine removed, in the aggregate, 72 percent (492) of 681 *Uncinaria* and 23 percent (181) of 788 *Ancylostoma* from 10 dogs. The only unfavorable reaction was vomition which occurred in 4 of 5 dogs that received the largest dosage. One animal in the latter group was given a tranquilizer pill about an hour before treatment, and in this instance the full dose of piperazine was retained. The adverse effect of vomition on the action of the chemical in this group is not evident from the aggregate data because most of the *Uncinaria* recovered after treatment were obtained from 1 dog in which emesis did not occur for at least 4 hours.

Vomition occurred in all of the 6 dogs that were given the 100-mg. dosage, although this was delayed for about 7 hours in 1 animal that received a tranquilizer pill before treatment. The data clearly show that the efficiency of the drug was substantially impaired in all except this animal.

The action of piperazine against *Uncinaria* was quite variable, particularly in the higher dosage groups. This variation was probably attributable, at least in part, to vomition, which seemed to be influenced by the method of dosing. Emesis usually occurred within an hour when the chemical was given in capsules, but it was delayed for at least 3 or 4 hours when the drug was mixed with feed. The administration of a tranquilizer apparently prevented, or markedly delayed, vomition in two instances, although the number of trials was too limited to permit definitive interpretations in this regard.

The performance of piperazine against *Ancylostoma* was not impressive in any of the dosages employed. The maximum efficacy achieved, 41 percent, was obtained in a single trial in which the drug was given at the rate of 100 mg. per pound of body weight, the largest dosage employed.

With the exception of vomition, which was unaccompanied by nausea, depression, or inappentence, piperazine was well tolerated by the test animals; and no lesions were observed at necropsy that could be attributed to the action of the drug. Moreover, there was no evidence that either of the compounds used in these trials was more effective or better tolerated than the other.

Although the data show that piperazine is more effective against *Uncinaria* than *Ancylostoma*, they do not show that the chemical is uniformly reliable against the former. Moreover, because *Uncinaria* occurs infrequently in dogs and cats in the United States, it is not likely that piperazine will attain sig-

**Trilafon (Schering Corporation).

TABLE 1.—Data on efficacy of piperazine against *Ancylostoma caninum* and *Uncinaria stenocephala* in dogs.

Number of Dogs	Chemical	Dosage (mg./lb.)	Parasites	Removed	Left	Efficacy (Percent)
2	Piperazine sulfate	25	Ancylostoma	7	63	10
			Uncinaria	106	64	62
2	Piperazine sulfate	50	Ancylostoma	21	105	17
			Uncinaria	282	105	73
1 **	Piperazine citrate	50	Ancylostoma	25	116	18
			Uncinaria	3	4	43
4 *	Piperazine sulfate	75	Ancylostoma	25	158	14
			Uncinaria	86	15	86
1 **	Piperazine citrate	75	Ancylostoma	103	165	38
			Uncinaria	15	1	94
5 *	Piperazine sulfate	100	Ancylostoma	21	173	11
			Uncinaria	99	316	21
1 * **	Piperazine citrate	100	Ancylostoma	41	60	41
			Uncinaria	6	0	100

*Vomited.
**Medication preceded by tranquilizer pill.

nificant status as a general anthelmintic for small animals in this country. The chemical should be very useful, however, in large roundworm infections. Piperazine may prove to be helpful also in parasite control programs for foxes raised in captivity, because *Uncinaria* is a common hookworm in these animals.

SUMMARY

In critical trials with 16 dogs, piperazine, in dosages ranging from 25 to 100 mg. per pound of body weight, exhibited significantly greater action against *Uncinaria stenocephala* than *Ancylostoma caninum*. The chemical was not uniformly reliable against either species, however, probably because of the high incidence of vomition associated with the larger dosages. The usefulness of piperazine in hookworm infections appears to be limited to those animals and areas in which *Uncinaria* is the predominant species.

LITERATURE CITED

BRADLEY, R. E., CRIMMENS, L. T., and ZWEIGART, T. F. 1956. Preliminary studies on the anthelmintic properties of piperazine citrate in dogs. J. Amer. Vet. Med. Assn. 128: 393-394.

COLGLAZIER, M. L., and ENZIE, F. D. 1951. Notes on caricide as an anthelmintic for cats and dogs. Proc. Helminth. Soc. Wash. 18: 50-52.

GUILHON, J. 1951. Un nouvel anthelminthique: Le diéthylène damine. Bull. Acad. Vét. France 24: 243-245.

KOTARBA, C., MARKIEWICZ, Z. MARKIEWICZ, K., and STANKIEWICZ, W. 1956. Zas tosowanie piperazyny do odrobaczania psów i swin. (Use of piperazine as a vermifuge for dogs and pigs.) Wiadom. Parazytol. 2: 145-147. Polish text; English sum.

MANN, P. H., HARFENIST, M., and DE BEER, E. J. 1955. The effectiveness of piperazine citrate against intestinal helminths of the cat and dog. J. Parasitol. 41: 575-578.

SLOAN, J. E. N., KINGSBURY, P. A., and JOLLY, D. W. 1954. Preliminary trials with piperazine adipate as a veterinary anthelmintic. J. Pharm. and Pharmacol. 6: 718-724.

Meningeal Tumors of the Newt Associated with Trematode Infection of the Brain

EDWARD W. LAUTENSCHLAGER*

Examination of 325 specimens of the newt, *Triturus viridescens viridescens* (Rafinesque) collected from Albemarle County and Giles County, Virginia, disclosed 40 animals infected with the strigeid metacercaria, *Diplostomulum sp.* The metacercariae were found unencysted in the brain case and brain of the host. Macroscopic and microscopic examinations of the brains of these infected forms have disclosed distinct histopathological conditions in 6 of the 40 infected animals.

Schlumberger and Lucké (1948), in their review of tumors of amphibians, reported only 7 cases of urodele tumors, all of the skin or testes. Smith and Coates (1939) reported a correlation between a fibro-epithelial tumor of the marine turtle, *Chelonia mydas* (L.) and the presence of trematode eggs. Smith, Coates and Nigrelli (1941) reported a papillomatous hyperplasia of the mucous membrance of the gall bladder of the marine turtle as associated with concurrent infection with *Rhytidodoides similis* Price.

Kelley (1934) in describing *Diplostomulum trituri* from the brain case and eyes of the newt does not record any pathological abnormalities of the host. Her report, however, does not indicate that any examination was made for pathological conditions.

Hoffman (personal communication) and Hoffman and Hundley (1957) have clearly demonstrated, by a series of experimental infections, the development of a tumor-like hyperplastic cyst of the brain of the stickleback, *Eucalia inconstans,* as a result of parasitism by *Diplostomulum baeri eucaliae.*

MATERIALS AND METHODS

Three hundred newts were obtained from several ponds in Albemarle County, Virginia, and 25 animals were collected from Mountain Lake, Giles County, Virginia.

The animals were anesthetized by immersion in MS-222 (tricaine methanesulfonate), the head was removed by a transverse cut just posterior to the angle of the jaw and entry made to the brain case by cuts through the ventral skull. The head was immersed in either Holtfreter's solution or amphibian saline and examined with the aid of a Bausch and Lomb dissecting microscope. Metacercariae in the brain case rapidly made their way to the exterior and moved about freely in the medium. The brain was then carefully removed from the case and all surfaces examined. The brain of all infected animals, every tenth uninfected brain, and 10 normal heads were immediately fixed in 9 parts Susa and 1 part saturated picric acid. This was followed with Cellosolve, amyl acetate, and paraffin embedding procedure.

The materials were sectioned at 6-10 microns, stained with Harris hemotoxylin, and counterstained with eosin or Orange G. The periodic acid-Schiff reaction (PAS) and a histochemical procedure for organic iron (Glick, 1949) were employed on selected specimens. Slides were cleared in xylene and toluene and mounted with clarite.

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OBSERVATIONS

In the case of 21 of 40 infected animals examined, metacercariae were noted, in varying numbers, ranging from 5 to 35, within the ventricles of the brain, submeningeal, and free in the brain case. Metacercariae were found both in the submeningeal and the brain case, but not in the ventricles of the remaining 19 infected animals. The metacercariae were not encysted and could be observed in vigorous motion in all locations.

The first apparent pathological condition to be noted is a rather marked increase in the size of the ventricles of the cerebral hemispheres (Fig. 2) as compared with the normal condition as seen in Fig. 1. Also apparent in the infected brain is the absence of the choroid plexus in the ventricles, which is clearly seen in the normal specimen.

In all cases of infection there is a hyperplasia of the meninges and the vascular plexus. This varies in degree and there is no correlation between the number and location of metacercariae and the degree of pathology.

In one case, a particularly large, distinct tissue mass was noted on the dorsal and dorso-lateral surfaces of the cerebral hemispheres (Figs. 3 and 4). Associated with the tissue mass, which has been characterized pathologically as comparable to a benign meningioma of the human, is noted the previously mentioned pathology of hyperplasia of the meninges and choroid plexus. In all cases there is also an increase in the pigment elements of the meninges.

There was a positive PAS reaction in varied regions of the meninges and choroid plexus of infected animals, as well as the presence of PAS positive cells in the large tumor mass described above. The metacercariae showed such a strongly positive PAS reaction that clear discrimination of cell outlines was impossible.

The cells of the ependymal layer of those brains with intraventricular parasites showed an increase in cell size, and in one case, the presence of brown cytoplasmic pigmentation was clearly noted. Histochemical procedures for organic iron were carried out on this specimen with negative results.

In 5 of the 40 infected animals, 1 to 2 metacercariae were noted in the intercerebral space as seen in Fig. 3. In all 5 cases, the metacercariae were positioned with the anterior end toward the ventral side of the brain.

CONCLUSIONS

At the present time there is no conclusive evidence, as based on experimental infection, that the tumorous conditions here reported are a direct result of the presence of the trematode. Correlation procedures would indicate a very strong possibility that such a definite relationship exists. In 325 newts examined, pathological conditions have been noted in only those animals which are parasitized by the trematode. The conclusion of relationship between the presence of the parasite and tumor formation is further supported by the recent experimental studies of Hoffman and Hundley (1957) in the formation of a hyperplastic tumor-like cyst of the brain of the fish as a result of metacercarial infection, and the reports of Smith and Coates (1939) and Smith, Coates, and Nigrelli (1941) associating tumorous conditions in the marine turtle with concurrent trematode infection.

It is suggested the excitatory cells may be of meningeal origin. The most generalized pathology is that of an increase in the thickness of the meningeal layer, with the possibility of metabolic changes as suggested by a positive PAS reaction in cells of the meninges of infected animals. It must also be

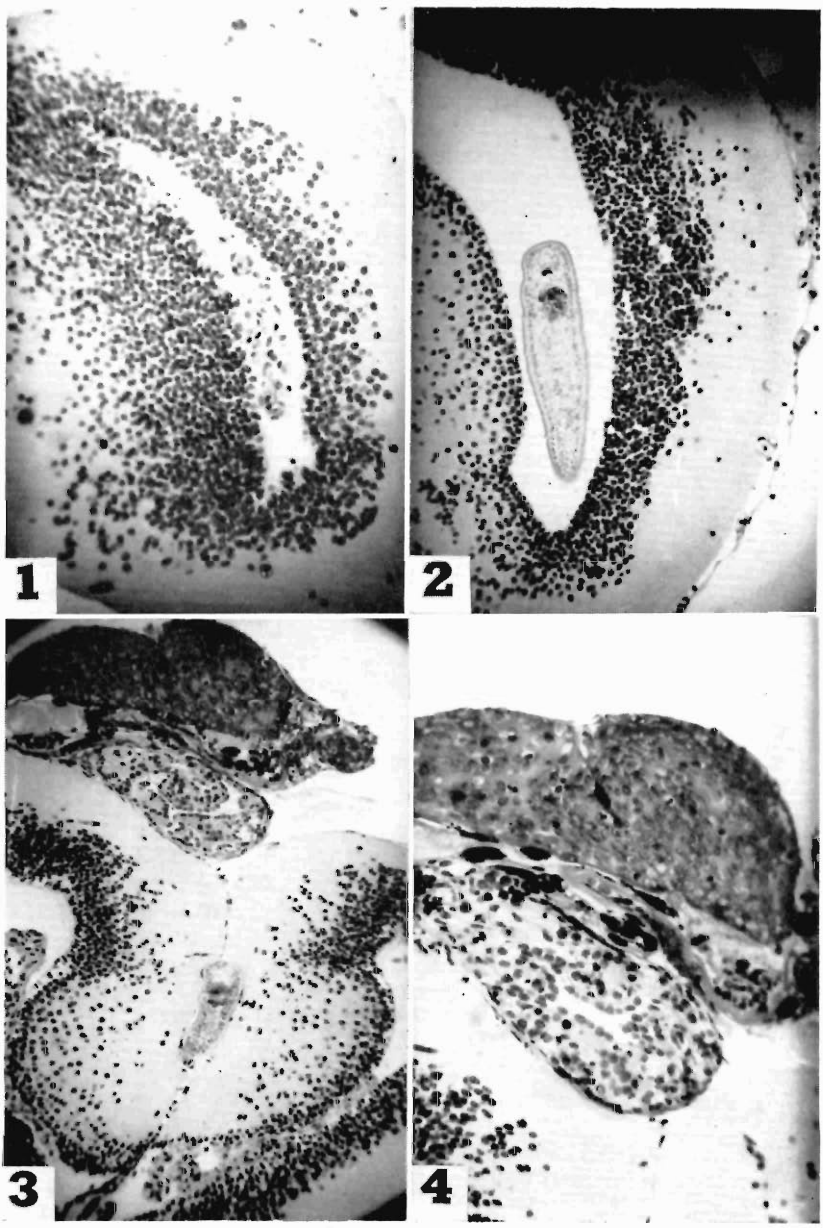

Figure 1. Cerebral hemisphere, uninfected newt. x.s.

Figure 2. Cerebral hemisphere with metacercaria in ventricle. x.s. approximately same level as Fig. 1.

Figure 3. Large tissue mass on dorsal aspect of brain. Metacercaria in intercerebral space. x.s.

Figure 4. Tissue mass seen in Fig. 3. Increase in pigment elements of meninges. Large vascular bundle ventral to tumor. x.s.

noted that there are abnormalities of the vascular system of the choroid plexus, as evidenced by an increase of capillary bundle mass and, in some cases, an increase in the diameter of vessels.

The brown pigmentation noted in the cytoplasm of the cells of the ependymal layer suggested the presence of haemoglobin, possibly resulting from destruction of the plexus. However, in view of negative results in histochemical procedures for organic iron, further investigation of this pigment material is necessary to establish its probable origin.

There is no noticeable gross effect of parasitism on the host. All infected newts so far examined have been at, or above, normal size. Definite conclusions in this regard can not be derived without study of a complete course of infection in a large group of newts.

LITERATURE CITED

HOFFMAN, G. L. and J. B. HUNDLEY. 1957. The life-cycle of *Diplostomulum baeri eucaliae* n. subsp. (Trematoda: Strigeida). Jour. Parasit. 43: 613-627.

KELLEY, R. S. 1934. The trematode parasites of *Triturus viridescens viridescens* (Rafinesque). Univ. Pitt. Bull. 31: 201-210.

GLICK, D. 1949. Techniques of histo- and cytochemistry. Interscience Publishers, Inc., New York.

SCHLUMBERGER, H. G. and B. LUCKÉ. 1948. Tumors of fishes, amphibians and reptiles. Canc. Res. 8: 657-753.

SMITH, G. M. and C. W. COATES 1939. The occurrence of trematode ova, *Hapalotrema constrictum* (Leared), in fibroepithelial tumors of the marine turtle, *Chelonia mydas* (Linnaeus). Zoologica 24: 379-382.

SMITH, G. M., C. W. COATES and R. F. NIGRELLI. 1941. A papillomatous disease of the gallbladder associated with infection by flukes, occurring in the marine turtle, *Chelonia mydas* (Linnaeus). Zoologica 26: 13-17.

Systematic Notes on the Monogenetic Trematodes*

WILLIAM J. HARGIS, JR.

The many publications on monogenetic trematodes during the past five years suggest an increasing interest in this group. That this interest is worldwide is evidenced by the large number of papers from the United States, Canada, Mexico, England, Germany, Sweden, India, and Russia. Most of the recent papers have been systematic, *viz.* Bychowsky (1951), Bychowsky and Nagibinia (1954), Bychowsky and Poliansky (1954), Caballero and Hollis (1955), Caballero, Hollis and Grocott (1953, 1954 and 1955), Chauhan (1953), Kaw (1950), Koratha (1955b), Malmberg (1956a), Manter (1955), Manter and Walling (1958), Millemann (1956), Monaco, Wood and Mizelle (1954), Ramalingham (1953), Reichenbach-Klinke (1954), Subhapradha (1951), Winter (1955), Wood and Mizelle (1957), and most of the papers by the present author. However, an increasing number of authors have given attention to other important phases of monogeneid biology. Llewellyn (1954, 1956a, 1956b and 1957a) and Johri and Smyth (1956) have studied micromorphology and micro-ecology; Euzet (1955), Bychowsky (1957), Llewellyn (1957b), Frankland (1955), Malmberg (1956b), and Turnbull (1956) have studied embryology and life cycles; and Bychowsky (1957), Hargis (1953b and 1957d), Koratha (1955a), Llewellyn (1957c), and Malmberg (1956b), have studied host-specificity.

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The obvious importance of care in the collection and identification of both hosts and parasites was stressed by Mayr (1957) and other participants in the first symposium on host-specificity among parasites of vertebrates. In their view, and correctly so, the validity of the conclusions is predicated upon careful and accurate host records. The inclusion of parasites from hosts whose identities are in doubt only serves to confuse host-specificity studies. Data from aquarium held fishes are generally unreliable unless special precautions have been taken to avoid contamination by foreign parasites. Data from fish markets are frequently spurious and those from sport and commercial catches must be examined critically before acceptance. Each investigator should know the detailed history of his host material from the time of capture to the collection of the parasites so that he can determine whether any opportunity for contamination by foreign flukes existed. It is particularly desirable to isolate hosts immediately after capture, but in mass-collecting this is seldom possible. Mizelle (1938) and Hargis (1953a) described several collection techniques useful for monogeneids.

Obviously care must be taken in the preparation of specimens for study. The recent techniques of Johri and Smyth (1956) and Malmberg (1956a) are noteworthy examples of careful work. Comparative studies are considerably improved by employment of a satisfactory relaxation technique prior to fixation.

Hargis (1954b) presented a detailed annotated list of terms useful in studies of morphology. In his discussion the terminology of clamp sclerites of the diclidophoroidid-type opisthaptor the terms used previously by Sproston (1954a), *viz.* ventral loop, dorsal loop elements and middle loop, were employed for the major transverse sclerites, but this is misleading. As Llewellyn (1956b and 1957a) has pointed out, in life the clamps project ventrally or away from the opishaptor with the open end, or gape, distal and the closed portion, the cup or base, proximal.

In contrast, clamps of mounted specimens are usually bent at the base by pressure of the cover slip so that the gape is directed posteriorly. The resulting alteration in direction and position of the clamp elements is artificial and can be confusing. In life, the "ventral loop" Llewellyn's (1957a) sclerite *a* or sclerite of the anterior (fixed) jaw, is actually posterior in position and should be called the anterior loop. The "dorsal loop elements" or Llewellyn's part of *a* in the posterior (fixed) jaw or valve are actually posterior in position and should be designated as the posterior loop elements. The term "middle loop," or Llewellyn's sclerite *d* (1956b) and *e* (1957a) of the movable posterior jaw is not ambiguous and can be retained. The term center piece or center sclerite is satisfactory to designate the sagittally-placed U or J-shaped clerite, "spring" of Sproston (1945b and 1946), sclerite *a* of Llewellyn (1956b) and sclerite *d* of Llewellyn (1957a). As Llewellyn (1956b and 1957a) and others have shown, the posterior movable half of the clamp (supported by the sclerites of the middle loop and including the posterior loop elements) opposes the anterior, immovable clamp half (supported by the sclerites of the anterior loop) when pinching host tissue between them.

Sproston (1945a) purported to show that the posterior loops (dorsal loops) of *Kuhnia scombri* and *K. minor* were complete and that this was the primitive condition, but Llewellyn (1957a) has shown that the posterior loop is not complete in *K. scombri*. As a result of his finding Llewellyn (1956) intimated that clamp structure might be over-rated as a taxonomic character,

stating, "If, then, clamp structure is of major phylogenetic importance, *Kuhnia, Mazocraes* and *Plectanocotyle* are closely related to each other." He further stated, "acceptance of this idea would, however, present a new problem; in general, among the Polyopisthocotylea, groups of nearly related parasites are restricted in their distribution to groups of nearly related hosts, *e.g.*, the Diclidophorinae on the Gadidae, and the Cyclocotylinae [= Choricotylinae] on the Sparidae; but the three closely related genera (?) *Mazocraes*, *Kuhnia*, and *Plectanocotyle* would then form a group parasitizing such widely divergent host families (Berg, 1947) as the Clupeidae (Clupeiformes), Scombridae (Perciformes, Scombroidei), and Triglidae (Perciformes, Cottoidei)." Despite Llewellyn's objections, worms belonging to the genera *Kuhnia* and *Mazocraes* have so many structures in common, *e.g.* clamp structure, cirral features, *etc.*, that, regardless of the systematic relationships of their hosts, they certainly seem to be related. Most other workers agree and place them in the same family. However, the position of *Plectanocotyle* is less clear, but its clamp structure and other features are definitely not mazocraeid (Hargis, 1955f). Indeed, the family Discocotylidae, in which *Plectanocotyle* is usually placed, is so heterogeneous that it is probably not a natural grouping and requires revision. Bychowsky (1957, see below) evidently recognized this problem and solved it by removing both *Plectanocotyle* and *Anthocotyle* from the family Discocotylidae and established a family for each, Plectanocotylidae Poche, 1925, and Anthocotylidae Bychowsky, 1951.

Actually it is not yet possible to fix the phylogenetic position of Mazocraeidae, which includes *Kuhnia*, with precision. Bychowsky (1957) placed the families Mazocraeidae and Hexostomatidae in a new suborder, Mazocraeinea. Mazocraeinea was placed in the order Mazocraeidea Bychowsky, 1957 and ranked after the order Chimaericolidea in his subclass Oligonchoinea, *cf. infra*. This solution cannot be evaluated until Bychowsky's work is translated.

It seems entirely reasonable to assume that the clamps and their sclerites arise ontogenetically in similar fashion from the same embryonic tissues throughout the entire group Monogenea. Indeed, it would be extremely unlikely that these structures arose *de novo* in the evolution of each family. Very probably all diclidophorideans are monophyletic and the structures which they share are phylogenetically significant. It is not fine details of tendon placement or "fair-lead" structure or even modifications of the accessory sclerites or center pieces, but the general, basic structure of the main sclerites which are systematically important—along with all other pertinent biological characters. Regardless of current criticisms it seems certain that the broad details of sclerite structure are taxonomic features of great value. In fact, the main clamp sclerites of all known diclidophoroidid groups can now be homologized. Following the lead of Sproston (1946), Hargis (1955h) suggested in detail that the aberrant sucker-like clamps of *Choricotyle* spp. [=*Cyclocotyla*] and *Pedocotyle* spp. are phyletically homologous to those of *Tagia equadori* which, in turn, are homologous to discocotylid, microcotylid and gastrocotylid types. The same scheme, but slightly modified, can be applied to the clamps of *Diclidophora* spp. Hargis (1957c) also pointed out that the highly aberrant sclerites of Hexostomatidae are probably homologous to those of other diclidophoroidids. Recognition of these homologies allows the most aberrant types, *Choricotyle* and *Hexostoma*, to be fitted into the system and the entire superfamily can thus be integrated. But details remain to be clarified and the primitive type has yet to be fixed. (It is possible that

the clamps of Chimaericolidae spp. whose sclerites can also be homologized, actually represent or are similar to the so-called "primitive-type" of clamp. However, further studies will be required to resolve the question. Clamp structure data should never be employed alone in making systematic decisions of a supraspecific nature but should always be accompanied by data of other external structures and all internal organs.

Hargis (1955g) presented a detailed discussion of the features of taxonomic significance and pointed out that the anchors and hooks of both larvae and adults may be used taxonomically. Other embryonic structures, *e.g.* eye spots, number of larval hooks and anchors, *etc.*, may be useful, but, as students of digenetic trematodes discovered some time ago, care must be exercised in employing larval characters alone in devising a system. Few monogeneid larvae, about 33-35, have been studied and the taxonomic importance of embryological characters have yet to be evaluated. Recently Malmberg (1956b) studied the excretory system of gyrodactylid larvae in some detail and his results seem to affirm the trematode affinities of monogeneids. The arrangement of the excretory structures may be useful in the systematics of the group.

Llewellyn (1956a) studied the micro-ecology and, especially, the correlation between microhabitat and certain features of body shape in some asymmetrical monogeneids. He pointed out that, in general, the direction of asymmetry was related to which gill chamber of the host the worms inhabited. Hargis (1956c and 1957b) noted that in many asymmetrical gastrocotylids and axiniids the direction of lateral asymmetry varied right or left, but the right-left orientation of the internal organs was always the same. It is possible that in these large asymmetrically-inclined worms, the unilateral impact (direction of the greatest force) or some correlated phenomenon of the gill ventilating current in some way stimulates one side to develop differently than the other. It is also possible that the direction (side) of asymmetry is predetermined genetically and that those not 'pre-adapted" for a particular side of the host's branchial apparatus may be dislodged by currents with which they are not equipped to cope. Although the former possibility is the more likely, the plasticity permitting such variable ontogenetic development is probably genetically controlled. In other words, the original development of the asymmetrical tendencies of certain microtylids and gastrocotylids are probably genetically determined because all known specimens of asymmetrical species are asymmetrical, and all of a normally symmetrical species are symmetrical. Although we do not know whether a normally asymmetrical fluke would develop symmetrically if it were experimentally fixed in such a way that the current flow would be equal on both sides, it probably would not. These postulations on the effects of unidirectional water currents on asymmetrical growth do not account for other types of monogeneid asymmetry such as those of *Anthocotyle* whose anteriormost clamps are much larger in size than the last three clamps of each side, or of the *Tagia*-complex whose anteriormost pair of clamps are often reversed 180° and whose posteriormost pair of clamps are often different in shape from the anterior three pairs. There is also a large group of species of several families which develop noticeable dorsoventral asymmetry. In fact, the body of *Amphipolycotyle chloroscombrus* Hargis, 1957 is not only laterally asymmetrical, but the clamps of one side are very different in shape as well as size than those of the other. Because of their general constancy these types of asymmetry, too, are probably genetically controlled and systematically significant.

SYSTEMATICS

The taxonomy of the order Monogenea is in a state of flux. Although most of the known groups probably represent natural genetic entities, new species and supraspecific taxa are being constantly added. This necessitates constant review in order to make realistic alignments as they are required.

The systematic arrangements of Price (1936 to 1943b), Sproston (1946) Chauhan (1953), Palombi (1949), Hargis (1954a to 1957b) and others are readily available, but the more recent work of Bychowsky (1957) is not. Because Bychowsky's system represents a significant departure from current concepts it is presented here.

CLASS—Monogenoidea (van Beneden) Bychowsky, 1937

SUBCLASS—Polyonchoinea, Bychowsky, 1937

A. Order—Dactylogyridea Bychowsky, 1937

1. Suborder—Dactylogyrinea Bychowsky, 1937
   a. Family—Dactylogyridae Bychowsky, 1933
      1. Subfamily—Dactylogyrinae Bychowsky, 1933
      2. Subfamily—Ancyrocephalinae Bychowsky, 1937
      3. Subfamily—Linguadactylinae Bychowsky, 1957
   b. Family—Diplectanidae Bychowsky, 1957
      1. Subfamily—Diplectaninae Monticello, 1903
      2. Subfamily—Rhamnocercinae Monaco, Wood and Mizelle, 1954
   c. Family-Protogyrodactylidae Johnston et Tiegs, 1922
   d. Family—Calceostomatidae (Parona and Perugia, 1890) Price, 1937
2. Suborder—Monopisthocotylinea (Odhner, 1912) Bychowsky, 1937
   a. Family—Monocotylidae Taschenberg, 1879
      1. Subfamily—Monocotylinae Gamble, 1896
      2. Subfamily—Dasybatotreminae Bychowsky, 1957
      3. Subfamily—Calicotylinae Monticelli, 1903
      4. Subfamily—Merizocotylinae Johnston and Tiegs, 1922
   b. Family—Loimoidae Bychowsky, 1957
   c. Family—Dionchidae Bychowsky, 1957
   d. Family—Capsalidae Baird, 1853
      1. Subfamily—Capsalinae Johnston, 1929
      2. Subfamily—Megalocotylinae Bychowsky, 1957
      3. Subfamily—Trochopodinae (Price, 1936) Sproston, 1946
      4. Subfamily—Entobdellinae Bychowsky, 1957
      5. Subfamily—Encotyllabinae Monticelli, 1892
      6. Subfamily—Nitzschiinae Johnston, 1934
   e. Family—Aconthocotylidae Price, 1936
      1. Subfamily—Acanthocotylinae Monticelli, 1903
      2. Subfamily—Enoplocotylinae Tagliani, 1912
   f. Family—Microbothriidae Price, 1936

B. Order—Tetraonchidea Bychowsky, 1957
   a. Family—Tetraonchidae Bychowsky, 1937
   b. Family—Amphibdellatidae (Carus, 1885) Bychowsky, 1957
   c. Family—Tetraonchoididae Bychowsky, 1951
   d. Family—Bothitrematidae Bychowsky, 1957

C. Order—Gyrodactylidea Bychowsky, 1937
1. Suborder—Gyrodactylinea Bychowsky, 1937
a. Family—Gyrodactylidae (van Beneden et Hesse, 1863) Cobbold, 1864
2. Suborder—Polyopisthocotylinea (Odhner, 1912) Bychowsky, 1937
a. Family—Polystomatidae (Carus, 1863) Gamble, 1896
b. Family—Sphyranuridae Poche, 1925

SUBCLASS—Oligonchoinea Bychowsky, 1937
A. Order—Diclybothriidea Bychowsky, 1957
a. Family—Diclybothriidae Bychowsky et Gussew, 1950
b. Family—Hexabothriidae Price, 1942
B. Order—Chimaericolidea (Brinkmann, 1952) Bychowsky, 1957
a. Family—Chimaericolidae Brinkmann, 1942
C. Order—Mazocraeidea Bychowsky, 1957
1. Suborder—Mazocraeinea Bychowsky, 1957
a. Family—Mazocraeidae Price, 1936
b. Family—Hexostomatidae Price, 1936
2. Suborder—Discocotylinea Bychowsky, 1957
a. Family—Discocotylidae Price, 1936
1. Subfamily—Discocotylinae Price, 1936
2. Subfamily—Diplozooninae Palombi, 1949
b. Family—Anthocotylidae Bychowsky, 1957
c. Family—Plectanocotylidae Poche, 1925
d. Family—Diclidophoridae Fuhrmann, 1928
e. Family—Microcotylidae Taschenberg, 1879
f. Family—Protomicrocotylidae Poche, 1925
g. Family—Gastrocotylidae Price, 1943

This system cannot be evaluated in detail because the supporting evidence has not been translated from the Russian. Certain items are treated separately below. It will be noted that Bychowsky used no superfamilial or superordinal groupings.

It was inevitable that some systematic duplications between the work of the present author and that of Koratha (1955a and b) should have occurred since both collected their material in the northern Gulf of Mexico at the same time. It seems desirable to point out the duplications, errors and oversights of both as well as call attention to apparent errors in pertinent works of other authors.

Order Monogenea Carus, 1863

There is much uncertainty concerning the proper rank to be accorded this taxon. Most common placement is ordinal but several digeneid specialists, *e.g.* Faust and Tang (1936) and LaRue (1957), and Chauhan (1953), who has considerable experience in both groups, employ is as a subclass. Bychowsky (1937 and 1957) considered it of class rank. It is probable that Monogenea and Digenea are more than ordinal in importance but whether they are of class rank is questionable. The present author believes sub-class rank justifiable at this time.

Chauhan (1953) was content to leave most of the subordinate taxa as placed by Sproston (1946) and Price (1936 and 1943b). Bychowsky, however, proposed two new subclasses, Polyonchoinea and Oligonchoinea, and numerous new orders. Bychowsky's drastic revisions will have to be studied

in detail before being accepted. The lower taxa should probably be re-aligned to the subclass ranking of Monogenea. Bychowsky's system could have easily been fitted in to the more commonly accepted subclass concept, if he had employed the categories superorder and superfamily.

Suborder Monopisthocotylea Odhner, 1912
Superfamily Gyrodactyloidea Johnston and Tiegs, 1922
Family Gyrodactylidae Cobbold, 1864

Genus *Gyrodactylus* Nordmann, 1832: Although several authors, Wood and Mizelle (1957), Malmberg (1956b), and Turnbull (1956), have recently published on the genus *Gyrodactylus*, its systematic situation is far from satisfactory. For example, several of the early reports of *G. elegans* made on this continent have never been verified and it is almost certain that the North American *G. elegans*-complex actually involves several species. In addition, many workers are content with very small differences in anchor, hook and haptoral bar shapes as criteria for new species. Until we have better knowledge of the range of infraspecific variability of these structures, we must recognize this possible weakness in the systematic foundations of this family.

Family Dactylogyridae Bychowsky, 1933
Subfamily Tetraonchinae Monticelli, 1903

The above criticisms apply to many of the species in this subfamily.

Genus *Amphibdelloides*, Price, 1937: Whether Bychowsky is justified in re-instating the family Amphibdellatidae Carus 1885 for this small group of species is doubtful. The genus currently contains *A. maccallumi* (Johnston and Tiegs, 1922) Price, 1937, from *Tetranarce occidentalis* (Storer) at Woods Hole, Massachusetts, and *A. narcine* Hargis, 1955, from *Narcine brasiliensis*, in the Gulf of Mexico. Both Price (1937) and Sproston (1946) give *Squalus acanthias* as a second host for this parasite, but unless Price obtained later unpublished records from MacCallum or some other source, this host record appears to be in error because MacCallum (1916) listed only *Tetranarce occidentalis* as the host. Sproston took her record from Price. Both parasites are very much alike.

Recently Alexander (1954) redescribed *A. maccallumi* from *Torpedo californica* Ayres from California. Alexander's species may be a new one, for the hosts are certainly discontinuously distributed. Actually all three flukes are similar and a detailed study of the variability of their taxonomic characters would be interesting because of the possible phylogenetic and zoogeographic significance of the host-parasite data. If the hosts are discontinuously distributed and specifically distinct yet the parasites nearly identical or very similar it may be possible to derive some notions concerning the rate of speciation in this monogeneid group and interrelationship of the hosts.

Superfamily Diplectaninae Monticelli, 1903

Genus *Rhamnocercus* Monaco, Wood and Mizelle, 1954: It is doubtful that the subfamily grouping, *Rhamnocercinae* Monaco, Wood and Mizelle, 1954 is justified; however, the genus *Rhamnocercus* appears to be sound. Hargis (1955c) erroneously gave *Pedunculospina* Hargis, 1954, *n. nud*, as a synonym of *Rhamnocercus*. Actually Hargis's (1954) publication was only an abstract of a microfilmed dissertation and because the new generic and subfamilial names were not supported by adequate descriptions all were *nomina nuda*

until redescribed. In the meantime, Monaco, Wood and Mizelle (1954) erected and published *Rhamnocercus* for another species of the same genus and *Pedunculospina* became unavailable. Bychowsky (1957) placed their subfamily grouping in his new family Diplectanidae.

Genus *Rhabdosynochus* (Mizelle and Blatz, 1941) Hargis, 1954: *Rhabdosynochus* was first described as tetraonchid, but because of its diplectanid characters, Hargis (1954a and 1955c) transferred it to Diplectaninae. Because he was evidently unaware of its diplectanid nature, as were the original authors, Bychowsky (1957) erroneously placed this genus in his subfamily Ancyrocephalinae, family Dactylogyridae. Interestingly, he separated certain tetraonchinid species from the ancyrocephalids and placed them in the family Tetraonchidae Bychowsky, 1937, order Tetraonchidea Bychowsky, 1957. That such wide separation of the very similar diplectanimids, ancyrocephalids, and tetraonchinids is justified is doubtful.

Superfamily Capsaloidea Price, 1936
Family Monocotylidae Taschenberg, 1879
Subfamily Dionchinae Johnston and Tiegs, 1922

Bychowsky (1957) assigned family rank to this aggregation in recognition of the great differences between it and other monocotylids. Recent work of the present author suggests that this separation is justifiable.

Genus *Dionchus* Goto, 1895: Hargis (1955e) and Koratha (1955f) described *Dionchus rachycentris* Hargis, 1955, from the gills of *Rachycentron canadus* in the northern Gulf of Mexico. According to communications from the editorial offices of both publications, the issue in which Hargis' description appeared was distributed (August) before that including Koratha's (September); therefore, the name *D. rachycentris* has priority over *D. hopkinsi* Koratha, 1955, which becomes a synonym.

Frankland (1955) pointed out that the terminal filaments of the egg capsules of most monogeneids do not seem to function primarily to anchor the capsules to the hosts' gills as has been so often asserted by others. For many species this may be true, but the two dionchinids, *D. rachycentris* and *D. remorae*, actually wrap the main filaments of their egg case clusters entirely around the gill filament in a permanent loop. In order to remove these egg capsule clusters, which appear like minute bunches of grapes, from the gills it is necessary to either sever the main filament loop of the egg cluster or slip it off the end of the gill filament (Hargis 1955e). *Entobdella corona* is often seen to clasp clusters of egg capsules by the expanded distal portions of their terminal filaments within the muscular pad of the genital atrium. The survival value and possible evolutionary significance of such arrangements are obvious.

Subfamily Loimoinae Price, 1936, *sensu* Hargis, 1955

Chauhan and Bhalerao (1945) transferred Loimoinae from the family Monocotylidae to Microbothriidae because of the lack of haptoral septa, different numbers of testes, aberrant nature of the ovary and the supposedly microbothriid-type prohaptors. This arrangement has merit, but because Microbothriidae itself needs some systematic attention, the writer prefers to follow Sproston's (1946) arrangement and leave Loimoinae in Monocotylidae. Bychowsky (1957) separated the loimoinids from the microbothriids, but whether he was justified in erecting the new family Loimoidae for them is not known. Should Loimoidae Bychowsky, 1957 prove to be a valid family

grouping, it would apparently contain three known genera, *Loimos* MacCallum, 1917, *Loimoisina* Manter, 1944, both very similar, and *Loimopapillosum* Hargis, 1955.

Genus *Loimos* MacCallum, 1957: Hargis suggested that the genera *Loimos* and *Loimosina* Manter, 1944, are very similar and that the characteristics separating them are not of generic importance. This requires further elucidation. *Loimos scoliodoni* (Manter, 1938) Manter, 1944, from the gills of *Scoliodon terrae-novae* was reported from the Gulf of Mexico by Hargis (1955e) who took 19 specimens from two specimens of a single host species. Koratha (1955b) obtained six from two *S. terrae-novae* and one from six *Carcharhinus limbatus* at Port Aransas, Texas. Koratha obtained only one ectoparasite from six *C. limbatus* and Hargis found none on three hosts of the same species, but both authors took a total of 25 specimens from three *S. terrae-novae*. Thus *S. terrae-novae* is probably the typical host.

Loimos salpingoides MacCallum, 1917, a species which is very similar to *L. scoloiodoni,* was reported from *Carcharhinus obscurus* LeSueur, 1818, from Woods Hole, Massachusetts. *Loimos secundus* Chauhan and Bhalero, 1945, was originally collected from a preserved specimen of *Scoliodon sorrakowah* Cuvier, 1829. It is remotely possible that the specimen from *C. limbatus* of the Gulf of Mexico is a different species of *Loimos* and not *L. scoliodoni* as Koratha (1955b) reported. Nevertheless *Scoliodon* and *Carcharhinus* belong to the same family, Carcharhinidae (Bigelow and Schroeder, 1948), and this similarity of gill parasites probably reflects the close host relationships. Loimoinids have not been reported outside this single host family and seem to exemplify rigid host-specificity as defined by Hargis (1957d).

Suborder Polyopisthocotylea Odhner, 1912
Superfamily Polystomatoidea Price, 1936
Family Hexabothriidae Price, 1942
Subfamily Hexabothriinae Price, 1942, *sensu* Sproston, 1946

Hargis (1955g) obviously misspelled the subfamily name as Hexabothriidae.

Genus *Heteronchocotyle* Brooks, 1934: Llewellyn (1954) concluded that many polyopisthocotylid trematodes feed on the blood of their hosts, but was forced to rely on histochemical tests rather than actual observation of feeding or of ingested blood cells in the gut because no intact erythrocytes were observed in the gut contents of hundreds of worms even though crystals of pryidene haemochromogen, a chemical derivative of blood cells, were obtained later. However, he studied some stained sections of *Discocotyle sagitatta* in which the gut lumen contained "what appeared almost certainly to be partially digested red blood cells." It is interesting that five of six specimens of *H. leucas* in the present collection contain numbers of undigested, nucleated oval cells in their gut crura which are probably intact shark red blood cells.

Superfamily Diclidophoroidae Price, 1936
Family Mazocraeidae Price, 1936

Bychowsky (1957) created the new order Mazocraeidea from this group in which he placed all families assigned by Sproston (1946) and others to the superfamily Diclidophoroidae Price, 1936.

Genus *Clupeocotyle,* Hargis, 1955: *Clupeocotyle* was erected for *C. brevoortia* and *C. megaconfibula* Hargis, 1955 from the gills of *Brevoortia patronus* Goode, taken at Alligator Harbor, Florida. Koratha (1955b) described a similar worm, *C. lintoni* (Koratha, 1955) *n. comb.* [= *Diclidophora lintoni Koratha,* 1955], from *B. gunteri* found near Port Aransas, Texas. Although the host and the arrangement of the worm's oviduct are slightly different, it is probable that both Hargis and Koratha's flukes are conspecific. However, the type specimens of *C. lintoni* are so distorted and the description is so superficial that accurate comparison and identifications are impossible. The Port Aransas fluke should be carefully collected and redescribed. Should later study definitely establish the conspecificity of *C. lintoni* (Koratha, 1955) *n. comb.* and *C. brevoortia* Hargis, 1955, the former name would take precedence by virtue of prior publication.

Genus *Kuhnia* Sproston 1945: Yamaguti (1953) described *K. otolithis* from the gills of *Otolithus* sp. from Celebes. Hargis (1954) recombined this species with *Tagia,* but later (1955g) erroneously listed it as a *n. comb.* The proper designation should be *Tagia otolithis* (Yamaguti, 1953) Hargis, 1954 [= *K. otolithis* Yamaguti, 1953] because the recombinations of Hargis (1954) are valid even though the paper was merely an abstract.

Genus *Mazocraeoides* Price, 1936: *Mazocraeoides* contains four known species, *M. georgei* Price, 1936, *M. dorosomatis* (Yamaguti, 1938) Sproston 1946, *M. prashadi* Chauhan, 1950 and *M. opisthonema,* Hargis, 1955. Hargis (1955f) redescribed *M. georgei* Price, 1936 twice. One description was based on new specimens from *B. patronus* from the Gulf of Mexico and the other was from Linton's U. S. N. M. Helm. Coll. slide No. 35623 from *Pomolobus pseudo-harengus* (Wilson) and *P. mediocris* (Mitchell) from Woods Hole, Massachusetts. Separate descriptions were made because those from the Gulf differed noticeably but perhaps not specifically from the Woods Hole specimens, and the author did not wish to mix the two groups because specific separation might later be necessary. Material from collections currently being made in the North Atlantic should permit settlement of this problem. In any case, all known species occur solely on clupeids, an example of rigid supraspecificity.

Family Discocotylidae Price, 1936

Hargis (1956a) pointed out that Discocotylidae contains several patently unrelated groups. There is no doubt that Discocotylidae is not an homogeneous aggregation, but whether Bychowsky's (1957) new arrangement, see above, resolves the dilemma is questionable.

Genus *Tagia* Sproston, 1946. *Tagia* was amended by Hargis (1956a) to accommodate *T. micropogoni* Pearse, 1949, *T. bairdiella* Hargis, 1956, and *Tagia cupida* Hargis, 1956. He did not then know that after restudying *T. equadori* (Meserve, 1938) Sproston, 1946, the type species, from fresh material and the type specimen Caballero *et. al.* (1953) had already decided that Pearse's species were not even congeneric with *T. equadori.* Actually *T. equadori* and *T. micropogoni* differ considerably and Caballero *et. al.* (1953) are probably correct in their generic separation of the two populations. Thus Hargis' action may not have been realistic. But Caballero *et. al.* have evidently neglected to determine its proper generic affinities to date even though it fits their own grouping *Macrovalvitrema* Caballero and Hollis, 1955, fairly well. *T. micropogoni,* Pearse, 1949, *T. bairdiella,* Hargis 1956 and *T. cupida* Hargis, 1956, are closely related to each other and to Cabal-

lero's and Hollis' (1955) species and may belong to *Hemitagia* Sproston, 1946, or to one of the generic aggregations mentioned immediately below. But they will be left in the genus *Tagia* pending the outcome of current studies.

Caballero *et. al.* (1954) erected *Pterinotrema* for *P. macrostomum* from *Albula vulpes* (Linn.) and placed it in the family Microcotylidae Taschenberg, 1879. Later Caballero and Hollis (1955) also described the genus *Macrovalvitrema* for *M. sinaloense* from the gills of *Micropogon ectenes* Jordan and Gilbert and *Pterinotrematoides* for *P. mexicanum* from the same host. At the same time, the subfamily Pterinotrematinae was erected for these three similar genera. But even if *P. mexicanum* and *M. sinaloense* actually are distinct species they are almost certainly congeneric because the differences used to separate the two groupings are probably not generic in stature. Both are closely related to the species of Hargis (1956a), see above. Obviously this extremely interesting group needs further careful study.

It is not possible to agree with Caballero's and Hollis' (1955) placement of this complex of species in the family Microcotylidae. They are more closely related to Diclidophoridae Fuhrmann, 1928, than to the microcotylids. They will be left in Discocotylidae Price, 1936, *sensu* Hargis (1956) pending clarification. Hargis (1956a) was able to trace the probable evolutionary line of the diclidophoridid clamp-type from that of *Tagia*.

Family Diclidophoridae Fuhrmann, 1938, *sensu* Price, 1943

Subfamily Choricotylinae Sproston, 1946

Chauhan (1953) provisionally followed Dawes (1946) in accepting the subfamily Cyclocotylinae Price, 1943. We prefer to retain the arrangement of Sproston (1946) which is based upon a fairly well-known type genus, *Choricotyle*, until the status of the incompletely characterized *Cyclocotyla* Otto, 1923, is better known.

Genus *Chorocotyle* van Beneden and Hesse, 1863: Hargis (1955h) described *C. aspinachorda* from 50 specimens taken from the gills of *Orthopristis chrysopterus*. A few month earlier Koratha (1955b) described *Diclidophora caudalis* from the caudal fin of *Leiostomus xanthurus* using a single specimen. A study of the holotype specimen U. S. N. M. Helm. Coll. No. 54760 clearly showed Koratha's fluke to be a *Choricotyle* sp. and not a *Diclidophora* sp. The name should be *C. caudalis* (Koratha, 1955) *n. comb.* with *D. caudalis* Koratha, 1955, as a synonym. Examination of Koratha's type specimen, description and figures discloses that *C. caudalis* and *C. aspinachorida* may be synonymous. If so, *C. caudalis* has priority by virtue of prior publication. However, the single type specimen is so distorted and the original description is so inadequate that conspecificity cannot be definitely established. Therefore, both species are provisionally retained pending further study.

It is unlikely that either the host, *L. xanthurus*, or the location on the host, skin of caudal region, reported by Koratha (1955b) for this parasite is correct. The present writer examined 26 specimens of *L. xanthurus* from the northern Gulf of Mexico but no *Choricotyle* sp. was among them. However, of 18 *O. chrysopterus* examined, eight or 44 per cent yielded 34 specimens of *C. aspinachorda*. In many instances, both hosts were taken in the same net haul. If *C. caudalis* and *C. aspinachorda* are synonymous as the present writer suspects, then in all likelihood *O. chrysopterus* is the natural host and

Koratha's record of *L. xanthurus,* also from the northern Gulf of Mexico, is spurious. The fact that the worm was found on the skin, rather than the gills where most other *Choricotyle* sp. have been found, supports this suspicion. Both hosts should be carefully collected at Port Aransas and their parasites studied to determine if these two species are conspecific.

Family Microcotylidae Taschenberg, 1879

Genus *Microcotyle* van Beneden and Hesse, 1863: Hargis (1957a) described *M. pseudomugilis* from gills of *Mugil cephalus* and suggested on the basis of internal evidence that the worms which Koratha (1955b) identified as *Metamicrocotyla macracantha,* were actually *M. pseudomugilis* Hargis, 1957. But positive identification from Koratha's (1955b) descriptions and figures is impossible. To resolve this difficulty an effort should be made to carefully collect and restudy both flukes from *Mugil cephalus* at Port Aransas where Koratha worked.

Genus *Metamicrocotyla* Yamaguti, 1953: Though his worms were probably *Microcotyle pseudomugilis* Hargis, 1957, and not *Metamicrocotyle macracantha* as they were identified, see immediately above, Koratha (1955b) recognized that Alexander's (1954) species, *M. macracantha,* belonged in the genus *Metamicrocotyla* (Alexander, 1954). Koratha suggested that *Microcotyle cephalus* Azim, 1939, from Alexandria, Egypt, was metamicrocotylid in character, but Hargis (1954) had already recombined Azim's species with *Metamicrocotyla.* Koratha further suggested that *M. mugilis* Vogt, 1878, also is a *Metamicrocotyla* species, but the presence of a vagina automatically excludes it from this genus. Actually, *Metamicrocotyla* is not soundly established and requires further study.

Subfamily Axininae Monticelli, 1903, *sensu* Hargis, 1956

Genus *Axinoides* Yamaguti, 1938 *sensu* Hargis, 1956: *A. truncatus* Hargis, 1956, was taken from the gills of *Tylosurus raphidoma* (Ranzani) at Alligator Harbor, Florida. Caballero et. al. (1954) described *Axine resplendens* from *T. fodiator* from Panama. Both species are very similar and probably congeneric. Hargis (1956c) pointed out that the genera *Axinoides* and *Axine* Abildgaard, 1794, are very much alike. A careful study should be conducted to determine the validity of *Axinoides,* the most recently erected genus.

Family Gastrocotylidae Price, 1943

Subfamily Gastrocotylinae. Sproston, 1946, *sensu* Hargis, 1956

Chauhan (1945 and 1954), Dawes (1946), and Hargis (1956b), accepted Price's (1943b) family Gastrocotylidae despite Sproston's (1946) rejection. Llewellyn's (1957b) embryological evidence supports this acceptance. Chauhan (1953) was unaware that *Vallisia* spp. are gastrocotylid in nature and, therefore, included only the two subfamilies, Gastrocotylinae Sproston, 1946 and Priceinae Chauhan, 1953, in Gastrocotylidae. Priceinae, which was erected for Pricea Chauhan, 1945, differs from Gastrotylinae in the bilateral condition of the haptor and the presence of rib-like thickenings in the wall of the clamp. Chauhan (1953) included *Gastrocotyle* van Beneden and Hesse, 1863, *Pseudaxine* parona and Perugia, 1890, and *Vallisiopsis* Subhapradha, 1951, in Gastrocoylinae (Sproston, 1946) *sensu* Chauhan, 1953. (But the inclusion of *Vallisiopsis* in Gastrocotylinae is not realistic, see below.) *Amphipolycotyle* Hargis, 1957 also would seem to fit into Gastro-

cotylinae as delineated by Chauhan (1953). Priceinea includes *Pricea* Chauhan, 1945, *Lithidocotyle* Sproston, 1946, and *Thoracocotyle* MacCallum, 1913. If Priceinea is a valid subfamily grouping it should probably also contain *Gotocoyla* Ishii, 1936, *sensu* Hargis, 1956, and *Neothoracocotyle* Hargis, 1956. The positions of *Chauhanea* Ramalingham, 1953, which according to Ramalingham's (1953) diagnosis, has, "haptor bilateral asymmetrical, rib-like thickenings in the clamp capsule absent . . ," and *Scomberocotyle* Hargis, 1956, which is both asymmetrical and has wall sclerites, in this scheme are not clear. Actually this arrangement is not entirely satisfactory and it is hoped that the current interest in monogeneids eventually will result in clarification of the situation.

Ramalingham's (1952) description of six separate species of *Pricea* from the gills of *Cybium guttatum* collected at the same location is probably unrealistic because the taxonomic characters he employed were insignificant. In all probability he merely described normal infraspecific variability and most of his species are not valid.

Genus *Pseudaxine* Parona and Perugia, 1890: Hargis (1956b) suggested on the basis of internal evidence that *P. texana* Koratha (1955) from *Scomberomorus maculatus* at Port Aransas, Texas, was synonymous with *P. mexicana* Meserve, 1938, from the same host at Tangola-Tangola, Mexico. Later examination of Koratha's holotype U. S. N. M. Helm. Coll. No. 54758 showed that: (1) the specimen was folded anteriorly and much contracted, which accounts for the "blunt anterior end whose outline is almost horizontal . . ., and the (2) conspicuous transverse striations or ridges at the anterior part of the body"; (3) some of the clamps, of which the present author was only able to count 40, not "45," as described by Koratha, were clearly torn off rendering a clamp count of little value; (4) only one anchor, not "one pair," was seen; and (5) the body is so distorted that it is impossible to tell with certainty whether the "caeca unite in the opisthaptor." Thus all of Koratha's distinguishing characters are of extremely doubtful validity and *Pseudaxine texana* Koratha, 1955, is probably a synonym of *P. mexicana* Meserve, 1938.

Subfamily Vallisiinae Price, 1943, *sensu* Hargis, 1957

Bychowsky (1957) placed *Vallisia,* the type genus, and *Winkenthughesia* Price, 1943, in the family Anthocotylidae Bychowsky, 1957. Because the genera *Vallisia,* and *Winkenthughesia* are obviously gastrocotylids as indicated by their gastrocotylid-type clamps and other structural similarities, this placement is erroneous.

Genus *Winkenthughesia* Price, 1943: The type species *W. thyrsites* (Hughes, 1928) Price, 1943, seems definitely related to *Vallisia* by virtue of its general morphology and the presence of the extra clamp sclerites, which Hughes (1928) clearly figured. Figure 301, p. 427, of Bychowsky (1957) also clearly shows the extra clamp sclerites of *W. bramae* (Parona and Perugia, 1896) Bychowsky, 1957, which supports Bychowsky's placement of this species in *Winkenthughesia.* Synonyms for this species are: *Octobothrium bramae* Parona and Perugia, 1896; *Octocotyle bramae* (Parona and Perugia, 1896) St. Remy, 1898; *Kuhnia bramae* (Parona and Perugia, 1896) Sproston, 1946; and *Mazocraes bramae* (Parona and Perugia, 1896) Palombi, 1949.

The vallisiinid complex of genera probably should also include *Allodiscocotyla* Yamaguti, 1953 *sensu* Hargis, 1957, *Lethacotyle* Manter and Prince, 1953, *Protomicrocotyle* Johnston and Tiegs, 1922, *Pseudomazocraes* Cabal-

lero and Hollis, 1955, and *Vallisiopsis* Subhaphadha, 1951. It is possible that Vallisiinae, even though having definite gastrocotylid affinities, may deserve familial rank because of such features as the small number of clamps, odd body asymmetry, etc., but until further evidence is accumulated Vallisiinae will be left in the family Gastrocotylidae.

Genus *Pseudomazocraes* Caballero and Hollis, 1955: Because their specimens came from several different hosts from several areas and Caballero and Hollis (1955) noted certain morphological differences between the various geographical groups which may be systematically significant, the type species *P. monsivaisae* should be carefully re-examined. It may include at least two species.

SUMMARY

Recent world-wide developments in studies of the biology of monogeneid ectoparasites of fishes have been considered briefly. The need for careful collection and preparation of hosts and parasites intended for use in studies of phylogeny, host-specificity and zoogeography has been stressed. Certain confusing aspects in the terminology of clamp sclerites have been reviewed and a brief consideration of the phylogenetic implications of clamp morphology and other anatomical features has been presented. We have concluded that the clamp sclerites of Diclidophoroidea are phylogenetically important and, therefore, taxonomically useful.

Notes on asymmetry in the Monogenea have been presented with the conclusion that in all probability asymmetry in the Monogenea is genetically controlled. This genetic control either determines the specific direction of asymmetry or permits the plasticity of development which, under relatively constant stresses of the environment, results in regular development of certain types of asymmetry. Regardless of the superficial causes, all, except perhaps teratological, asymmetry is predetermined to a certain extent.

The interesting new systematic scheme of Bychowsky (1957) has been presented and partially discussed. A complete translation is necessary before more thorough analyses can be made. In addition, certain portions of the systematic arrangement employed in previous papers of this series have been revised and clarified and the unpublished U. S. N. M. Helminthological Collection Numbers of various species described in earlier installments have been listed, see appendix. The unsatisfactory systematic condition of *Gyrodactylus* and other members of the superfamily Gyrodactyloidea have been noted. New combinations have been made for *Clupeocotyle lintoni* (Koratha, 1955) [= *Diclidophora lintoni* Koratha, 1955] and *Choricotyle caudalis* (Koratha, 1955) [= *Diclidophora caudalis* Koratha 1955]. *Dionchus rachycentria* Hargis, 1955, from *Rachycentron canadus* has priority over *Dionchus hopkinsi* (Koratha, 1955) which is a synonym and *Pseudaxine texana* (Koratha, 1955) has been reduced to synomy with *P. mexicana* Meserve 1938. In addition, certain necessary alterations in the systematics of the subfamilies Gastrocotylinae and Vallisiinae of the family Gastrocotylidae have been made.

LITERATURE CITED

ALEXANDER, C. G. 1954. *Microcotyle macracantha* n. sp. a monogenetic trematode from the Gulf of California, with a redescription of *Amphibdelloides maccallumi* (Johnston and Tiegs, 1922) Price, 1937. J. Parasitol., 40: 279-283.

BERG, L. S. 1947. Classification of fishes both recent and fossil. J. W. Edwards, Ann Arbor. 517 pp.

BIGELOW, H. B. and W. C. SCHROEDER. 1948. Sharks. *In*: Fishes of the Western North Atlantic. Part I. Mem. Sears Found. for Marine Res. 1: 59-546.

BYCHOWSKY, B. E. 1937. Ontogenese und phylogenetische Beiziehungen der parasitischen Plathelminthes. (Russian text; German summary) Izvest. Akad. Nauk. SSSR, Seria. Biol. 4: 1353-1383.

BYCHOWSKY, B. E. 1951. *Tetraonchoides*—a new genus of monogenetic trematodes. Parasitol. Sborn. Akad. Nauk. SSSR 13: 29-34.

BYCHOWSKY, B. E. 1957. Monogenetic trematodes, their systematics and phylogeny. (In Russian) Acad. Nauk. SSSR: 1-509.

BYCHOWSKY, B. E. and L. F. NAGIBINIA 1954. New monogenetic trematodes from the Pacific ocean. Zool. Zhurnal, 33: 30-38.

BYCHOWSKY, B. E. and I. I. POLIANSKY 1954. Contribution to the knowledge of marine monogenetic trematodes of the family Gyrodactylidae Cobb. (In Russian) Trudy Zool. Inst. Akad. Nauk. SSSR 13: 91-126.

CABALLERO, y C. E. and M. BRAVO-HOLLIS 1955. Trematodes de peces marinos de aquas Mexicanas del Oceano Pacifico. VIII. Descripcion de tres nuevos generos de trematodos monogeneos. Anales del Inst. de Biol. 26: 89-115.

CABALLERO, y C. E., M. BRAVO-HOLLIS, and R. G. GROCOTT 1954. Helmintos de la Republica de Panama. XII. Descripcion de dos nuevos trematodes monogeneos, parasitos de peces marinos comestibles del oceano Pacifico del Norte. Ciencia 14: 81-86.

CABALLERO, y C. E., M. BRAVO-HOLLIS, and R. G. GROCOTT 1955. Helmintos de la Republica de Panama. XIV. Trematodos monogeneos y digeneos de peces marinos del oceano Pacifico del Norte, con descripcion de nuevas formas. Anales del Inst. de Biol. 26: 117-147.

CHAUHAN, B. S. 1945. Trematodes from Indian marine fishes. Part I. On some new monogenetic trematodes of the sub-orders Monopisthocotylea Odhner, 1912, and Polyopisthocotylea Odhner, 1912. Proc. Indian Acad. Sci. Section B. 21: 129-159.

CHAUHAN, B. S. 1953. Studies on the trematode fauna of India. Part 1. Subclass Monogenea. Rec. Indian Mus. 51: 113-208.

CHAUHAN, B. S., and G. D. BHALERO 1945. *Loimos secundus* (Monogenea, Trematoda) from the gills of the common Indian Dogfish (*Scoliodon sorrakowah*). Proc. Indian Acad. Sci. 22: 164-167.

DAWES, B. 1946. The trematoda. With special reference to British and other European forms. Cambridge U. Press. xvi-644.

EUZET, L. 1955. Larves gyrodactyloides nageantes de quelques trematodes monogenetiques de poissons marins. Bull. de la Soc. neuchateloise des Sci. nat. 78: 71-79.

FAUST, E. C. and C. C. TANG 1936. Notes on new aspidogastrid species, with a consideration of the phylogeny of the group. Parasitol. 28: 487-501.

FRANKLAND, H. M. T. 1955. The life-history and bionomics of *Diclidophora denticulata* (Trematoda: Monogenea). Parasitol. 45: 313-351.

HARGIS, W. J. JR. 1953a. Chloretone as a trematode relaxer, and its use in mass-collecting techniques. J. Parasitol. 39: 224-225.

HARGIS, W. J. JR. 1953b. Monogenetic trematodes of Westhampton Lake Fishes. III. Part 2. A discussion of host-specificity. Proc. Helminthol. Soc. Wash. 20: 98-104.

HARGIS, W. J. JR. 1954a. Monogenetic trematodes of some Gulf of Mexico fishes. Dissert. Abstr. 14: 1115-1116.

HARGIS, W. J. JR. 1954b. An annotated list of terms useful for studies of monogenetic trematodes. Mimeo. 1954: 1-6.

HARGIS, W. J. JR. 1955a. Monogenetic trematodes of Gulf of Mexico fishes. Part II. The superfamily Gyrodactyloidea. (Continued). J. Parasitol. 41: 185-193.

HARGIS, W. J. JR. 1955b. Monogenetic trematodes of Gulf of Mexico Fishes. Part I. The superfamily Gyrodactyloidea. Biol. Bull. 108: 125-137.

HARGIS, W. J. JR. 1955c. Monogenetic trematodes of Gulf of Mexico fishes. Part III. The superfamily Gyrocactyloidea (Continued). Quart. J. Fla. Acad. Sci. 18: 33-47.

HARGIS, W. J. JR. 1955d. Monogenetic trematodes of Gulf of Mexico fishes. Part IV. The superfamily Capsaloidea Price, 1936. Rev. Iberica. Parasitol. Vol. Extr. 1955: 1-16.

HARGIS, W. J. JR. 1955e. Monogenetic trematodes of Gulf of Mexico fishes. Part. V. The superfamily Capsaloidea. Trans. Amer. Micros. Soc. 74: 203-225.

HARGIS, W. J. JR. 1955f. Monogenetic trematodes of Gulf of Mexico fishes. Part VI. The superfamilies Polystomatoidea Price, 1936, and Diclidophoroidea Price, 1936. Trans. Amer. Micros. Soc. 74: 361-377.

HARGIS, W. J. JR. 1955h. Monogenetic trematodes of Gulf of Mexico fishes. Part IX. The family Diclidophoridae Fuhrmann, 1928. Ibid. 74: 377-388.

HARGIS, W. J. JR. 1956a. Monogenetic trematodes of Gulf of Mexico fishes. Part VIII. The superfamily Diclidophoroidea Price, 1936, (Continued). Proc. Helm. Soc. Wash. 23: 5-13.

HARGIS, W. J. JR. 1956b. Monogenetic trematodes of Gulf of Mexico fishes. Part XII. The family Gastrocotylidae Price, 1943. Bull. Mar. Sci. Gulf and Caribbean 6: 28-43.

HARGIS, W. J. JR. 1956c. Monogenetic trematodes of Gulf of Mexico fishes. Part XI. The family Microcotylidae Taschenberg, 1879, (Continued). Proc. Helm. Soc. Wash. 23, 153-162.

HARGIS, W. J. JR. 1957a. Monogenetic trematodes of Gulf of Mexico fishes. Part X. The family Microcotylidae Taschenberg, 1879. Trans. Amer. Micros. Soc. 74: 436-453.

HARGIS, W. J. JR. 1957b. Monogenetic trematodes of Gulf of Mexico fishes. Part XIII. The family Gastrocotylidae Price, 1943, (Continued). Trans. Amer. Micros. Soc. 76: 1-12.

HARGIS, W. J. JR. 1957c. The homologies of the clamp sclerities of *Hexostoma* (Hexostomatidae: Diclidophoroidea). J. Parasitol. 43, Section 2: 32 (Abstract 78).

HARGIS, W. J. JR. 1957d. The host-specificity of monogenetic trematodes. Exper. Parasitol. 6: 610-625.

HUGHES, W. K. 1928. Some trematodes on the gills of Victorian fishes. Proc. Roy. Soc. Victoria 41: 45-54.

JOHRI, L. N. and J. D. SMYTH. 1956. A histochemical approach to the study of helminth morphology. Parasitol. 46: 107-116.

KAW, B. L. 1950. Studies in helminthology: Helminth parasites of Kashmir. Part I. Trematoda. Indian J. Helminth. 2: 67-126.

KORATHA, K. J. 1955a. Studies on the monogenetic trematodes of the Texas coast. I. Results of a survey of marine fishes at Port Aransas, with a review of Monogenea reported from the Gulf of Mexico and notes on euryhalinity, host-specificity, and relationship of the Remora and Cobia. Proc. Inst. Marine Sci. 4: 233-250.

KORATHA, K. J. 1955b. Studies on the monogenetic trematodes of the Texas coast. II. Descriptions of species from marine fishes of Port Aransas. Proc. Inst. Mar. Sci. 4: 251-278.

LARUE, G. R. 1957. The classification of digenetic trematoda: A review and a new system. Exper. Parasitol. 6: 306-349.

LLEWELLYN, J. 1954. Observations on the food and the gut pigment of the Polyopisthocotylea (Trematoda: Monogenea). Parasitol. 44: 428-437.

LLEWELLYN, J. 1956a. The host-specificity, micro-ecology, adhesive attitudes, and comparative morphology of some trematode gill parasites. J. Mar. Biol. Ass. U. S. 35: 113-127.

LLEWELLYN, J. 1956b. The adhesive mechanisms of monogenetic trematodes: The attachment of *Pleetanocotyle gurnardi* (v. Ben. and Hesse) to the gills of *Trigla*. Ibid. 35: 507-514.

LLEWELLYN, J. 1957a. The mechanism of the attachment of *Kuhnia scombri* (Kuhn, 1829) (Trematoda: Monogenea) to the gills of its host *Scomber scombrus* L., Including a note on the taxonomy of the parasite. Parasitol. 47: 30-39.

LLEWELLYN, J. 1957b. The larvae of some monogenetic trematode parasites of Plymouth fishes. J. Mar. Biol. Assoc. U. K. 36: 243-259.

LLEWELLYN, J. 1957c. Host-specificity in monogenetic trematodes. First symposium on host specificity among parasites of vertebrates. I. U. B. S. and the Univ. of Neuchatel, 1957: 199-211.

MACCALLUM, G. A. 1916. Some new species of parasitic trematodes of marine fishes. Zoopathologica 1: 3-38.

MALMBERG, G. 1956a. On a new genus of viviparous monogenetic trematodes. Arkiv. for Zool. utgivel ar Kungl. Svenska Vetenskapsakad. Sev. 2, Band 10: 317-329.

MALMBERG, G. 1956b. Om forekomsten av Gyrodactylus pa Svenska Fiskar. Skvift. Utgiv. av severiges Fiskeniforenig Avsskvift. 1956: 19-76.

MANTER, H. W. 1955. Two new monogenetic trematodes from elephant fishes (*Callorhynchus*) from South Africa and New Zealand. Essays on the Nat. Sci. in honor of Capt. Allan Hancock.: 211-220.

MANTER, H. W. and G. WALLING. 1958. A new genus of monogenetic trematode (family Dicilidophoridae) from a New Zealand fish. Proc. Helminth. Soc. Wash. 25: 45-47.

MAYR, E. *et al.* 1957. Evolutionary aspects of host specificity among parasities of vertebrates. First symposium on host specificity among parasites of vertebrates. I.U.B.S. and Univ. of Neuchatel: 7-14.

MILLEMANN, R. E. 1956. Notes on the genus *Hexostoma* (Monogenea: Hexostomatidae) with a redescription of *H. euthynni* Meserve, 1938. J. Parasitol. 42: 316-319.

MIZELLE, J. D. 1938. Comparative studies on trematodes (Gyrodactyloidea) from the gills of North American fresh-water fishes. III. Biol. Mono. 17: 1-81.

MONACO, L. H., R. A. WOOD, and J. D. MIZELLE 1954. Studies on monogenetic trematodes XVI. Rhamnocercinae, a new subfamily of Dactylogyridae. Amer. Midl. Nat. 52: 129-132.

PALOMBI, A. 1945. I Trematode d'Italia. Parte I. Trematode monogenetici. Archiv. Zool. Italiano 34: 203-408.

PRICE, E. W. 1936. North American monogenetic trematodes. Geo. Wash. Univ. Bull. Summaries of Doctoral theses 1934-36: 10-13.

PRICE, E. W. 1937a. North American monogenetic trematodes. I. The superfamily Gyrodactyloidea. J. Wash. Acad. Sci. 27: 114-130.

PRICE, E. W. 1937b. North American monogenetic trematodes. I. The superfamily Gyrodactyloidea (continued). Ibid. 27: 146-164.

PRICE, E. W. 1938a. North American monogenetic trematodes. II. The families Monocotylidae, Microbothriidae, Acanthocotylidae, and Udonellidae (Capsaloides). Ibid. 28: 109-126.

PRICE, E. W. 1938b. North American monogenetic trematodes. III. The families Monocotylidae, Microbothriidae, Acanthocotylidae, and Udonellidae (Capsaloidea). Ibid. 28: 183-198.

PRICE, E. W. 1939a. North American monogenetic trematodes. III. The family Capsalidae (Capsaloidea). Ibid. 29: 63-92.

PRICE, E. W. 1939b. North American monogenetic trematodes. IV. The familly Polystomatidae (Polystomatoidea). Proc. Helmin. Soc. Wash. 6: 80-92.

PRICE, E. W. 1942. North American monogenetic trematodes. V. The family Hexabothriidae, N. n. (Polystomatoidea). Ibid. 9: 39-56.

PRICE, E. W. 1943a. North American monogenetic trematodes. VII. The family Discocotylidae (Diclidophoroidea). Ibid. 10: 10-15.

PRICE, E. W. 1943b. North American monogenetic trematodes. VI. The family Diclidophoridae (Diclidoporoidea). J. Wash. Acad. 33: 44-54.

RAMALINGHAM, K. 1952. Six new species of trematodes belonging to the genus *Pricea* Chauhan. Rec. Indian Mus. 49: 337-348.

RAMALINGHAM, K. 1953. A new genus of trematode (Chauhanea) from the gills of *Sphyraena acutipinnis Day*. J. Zool. Soc. Indian 5: 59-63.

REICHENBACH-KLINKE, H. 1954. Weitere mitteilung uber den Keimenparasiten *Diplozoon barbi* Reichenbach-Klinke (Trematoda: Monogenea). Zeit. fur. Parasiten Kunde. 16: 373-387.

SPROSTON, N. G. 1945a. The genus *Kuhnia* n.g. (Trematoda: Monogenea). An examination of the value of some specific characters, including factors of relative growth. Parasitol. 36: 176-190.

SPROSTON, N. G. 1945b. A note on the comparative anatomy of the clamps *in* the superfamily Diclidophoroidea (Trematoda: Monogenea). Ibid. 191-194.

SPROSTON, N. G. 1946. A synopsis of the monogenetic trematodes. Trans. Zool. Soc. London 25: 185-600.

SUBHAPRADHA, C. K. 1951. *Vallisiopsis contorta n.g.* and n. sp. and *Gastrocotyle indica* n. sp. monogenetic trematodes from marine fishes of the Madras Coast. Parasitol. 41: 162-165.

TURNBULL, E. F. 1956. *Gyrodactyle bullatarudis* n. sp. from *Lebistes reticulatus* Peters with a study of its life cycle. Canadian J. Zool. 34: 583-594.

WINTER, H. A. 1955. "*Capsala caballeroi* sp. n., Parasito de "*Sarda orientalis*," con un catalogo de los trematodos monogeneos de los peces del oceano Pacifico de los Americas. Rev. Brasil. Biol. 15: 9-32.
WOOD, R. A. and J. D. MIZELLE. 1957. Studies on monogenetic trematodes. XXI. North American Gyrodactylinae, Dactylogyrinae and a new host record for *Urocleidus dispar* (Mueller, 1936). Amer. Midl. Nat. 57: 183-202.
YAMAGUTI, S. 1953. Parasitic worms mainly from Celebes. Part 2. Monogenetic trematodes of fishes. Acta Med. Okayama. 8: 203-256.

APPENDIX

The following list of U. S. National Museum Helminthological Collection numbers was not available when the species were described (Hargis, 1955a and 1957b).

Suborder Monopisthocotylea Odhner, 1912
Superfamily Capsaloidea Price, 1936
Family Monocotylidae Taschenberg, 1879
Subfamily Monocotylinae Gamble, 1896

Neoheterocotyle inpristi Hargis, 1955, holotype, No. 38139.

Subfamily Dendromonocotylinae, Hargis, 1955.

Dendromonocotyle ocotodiscus Hargis, 1955, holotype, No. 38140.

Subfamily Merizocotylinae Johnston and Tiegs, 1922.

Thaumatocotyle longicirrus Hargis, 1955, holotype, No. 38141.
T. retorta Hargis, 1955, holotype, No. 38142.
T. pseudodasybatis, Hargis, 1955, holotype, No. 38143.

Subfamily Dionchinae Johnston and Tiegs, 1922.

Dionchus rachycentris Hargis, 1955, holotype and paratype, No. 38144.

Subfamily Loimoinae Price, 1936, *sensu* Hargis, 1955.

Loimopapillosum dasyatis Hargis, 1955, holotype and paratype, No. 38145.
Family Capsalidae Baird, 1853
Subfamily Benedeniinae Johnston, 1931
Benedenia posterocolpa Hargis, 1955, holotype, No. 38146.
Entobdella corona Hargis, 1955, holotype, No. 38147.
Suborder Polyopisthocotylea Odhner, 1912
Superfamily Polystomatoidea Price, 1936
Family Hexabothriidae Price, 1942

Subfamily Hexabothriinae Price, 1942, *sensu* Sproston, 1946

Dasyonchoctyle spiniphallus Hargis, 1955, holotype, No. 38148.
Heteronchocotyle leucas, holotype, No. 38149.
Squalonchocotyle inpristi, Hargis, 1955, holotype and paratype, No. 38150.
Superfamily Diclidophoroidea Price, 1936
Family Mazocraeidae Price, 1936
Clupeocotyle brevoortia Hargis, 1955, holotype, No. 37492.
C. megaconfibula Hargis, 1955, holotype, No. 37493.
Kuhnia brevoortia Hargis, 1955, holotype, No. 37491.
Mazocraeoides opisthonema Hargis, 1955, holotype, No. 37490.
Family Diclidophoridae Fuhrmann, 1928 *sensu* Price, 1943
Subfamily Choricotylinae Sproston, 1946
Choricotyle aspinachorda Hargis, 1955, holotype and paratype, No. 38151.
C. louisianensis Hargis, 1955, holotype, No. 38152.
Pedocotyle minima Hargis, 1955, holotype and paratype, No. 38153.
Family Microcotylidae Taschenberg, 1879
Microcotyle pseudomugilis Hargis, 1957, holotype, No. 38252.
M. pseudoheteracantha Hargis, 1957, holotype, No. 38251.
Metamicrocotyla macracantha (Alexander, 1954) Koratha, 1955, homotype, No. 38253.
Pyragraphorus hippos Hargis, 1957, holotype, No. 38254.
Family Gastrocotylidae Price, 1943

Subfamily Gastrocotylinae Sproston, 1946, *sensu* Hargis, 1956

Amphipolycotype chloroscombrus Hargis, 1957, holotype, No. 38255.

Subfamily Vallisiinae Price, 1943, *sensu* Hargis, 1957

Vallisia oligoplites Hargis, 1957, holotype, No. 38256.
Pseudomazocraes selene Hargis, 1957, holotype, No. 38257.

Trematode parasites of fishes from Egypt. Part I. *Basidiodiscus ectorchis*, n. gen., n. sp., and *Sandonia sudanensis* McClelland, 1957 (Paramphistomidae)*

JACOB H. FISCHTHAL and ROBERT E. KUNTZ

A collection of fish trematodes made between 1948 and 1953 by R. E. Kuntz, while a member of the U. S. Naval Medical Research Unit No. 3, Cairo, Egypt, contained specimens of Paramphistomidae recovered from *Synodontis schall* and *Mormyrus kannume*. The paramphistomids represented a new genus, and the genus *Sandonia* recently described by McClelland (1957) from the freshwater siluroid fishes, *S. schall* and *Distochodus niloticus*, collected from the River Nile, Khartoum, Tonga and Khor Barboi, Sudan. All worms of the present study were killed in hot water, then fixed overnight in FAA. After a change or two of alcohol they were placed in 70 per cent alcohol with 1–2 per cent glycerine. Whole mounts of immature and mature specimens as well as serial cross and sagittal sections were stained in either Harris hematoxylin or Mayer's paracarmine and mounted in balsam. The authors wish to express their appreciation to the London School of Hygiene and Tropical Medicine and to the British Museum (Natural History) for the loan of type slides of *Sandonia sudanensis*.

Basidiodiscus, n. gen.

DIAGNOSIS: Paramphistomidae. Body conical. Oral sucker terminal, with paired saccular pharyngeal diverticulae. Esophagus with posterior bulb Ceca simple, terminating short of posterior extremity. Acetabulum terminal, much like a pedestal, with many papilliform projections on its bottom. Testes 2, slightly oblique, widely separated, one extracecal at anterior third and other intercecal at middle of body. Cirrus pouch present. Genital pore mid-ventral, nearer to oral sucker than esophageal bulb. No genital sucker. Ovary mid-ventral, posttesticular and postcecal. Laurer's canal present. Uterine coils inter-, extra-, pre-, and postcecal. Vitelline follicles lateral, beginning at level of posterior testis or entirely posttesticular. Excretory vesicle with posterodorsal opening. Lymph system present. Intestinal parasite of fishes.

Basidiodiscus ectorchis, n. sp.

DIAGNOSIS: Body conical, anterior end rounded. Oral sucker terminal, mouth tilted slightly ventral. No oral papillae. Pharyngeal diverticulae 2, saccular, thick-walled, dorso-lateral to esophagus. Esophagus elongate, dorsal to anterior testis, terminating in muscular bulb at intestinal bifurcation; latter at level of posterior portion of anterior testis. Ceca simple, dorsal, terminating short of posterior extremity. Acetabulum terminal, much like a pedestal, tilted slightly ventral, with outer edge rolled down slightly; 12 prominent papilliform projections on its bottom, 11 in outer ring and 1 ven-

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trally within ring. Pigment granules of disintegrated cercarial eyespots distributed in all directions from around oral sucker to ends of ceca.

Testes 2, smooth, slightly oblique, widely separated, amphitypic. Anterior testis longitudinally elongate, at anterior third of body length, extracecal, mostly anterior to but slightly overlapping intestinal bifurcation ventrally. Posterior testis transversely elongate, at middle of body length, intercecal, occasionally overlapping cecum ventrally, entirely anterior to ends of ceca. Vas efferens of posterior testis arises from antero-dorsal margin, ascending body dorsal to anterior testis and ventral to esophagus. Vas efferens of anterior testis arises from antero-median margin, being joined immediately by other vas efferens. Vas deferens is inflated into seminal vesicle before entering cirrus pouch. Cirrus winding, opening into shallow genital atrium. Vas deferens, seminal vesicle, and cirrus pouch amphitypic, ascending from right if anterior testis on left or vice versa. Genital pore mid-ventral, nearer to oral sucker than esophageal bulb, anterior to or rarely under anterior portion of anterior testis, without genital sucker.

Ovary mid-ventral, posttesticular and postcecal near posterior end of body, elongate, smaller than either testis. Oviduct arises from postero-dorsal margin, passing dorsally to form short loop, then ventrally into ootype lying near posterior end of ovary and occasionally overlapping it slightly; oviduct expands into uterus a short distance farther. Ootype complex contains well-developed Mehlis' gland, common vitelline duct, and Laurer's canal contacting left margin of body. Uterine coils inter-, extra-, pre-, and postcecal, lying dorsal to posterior testis, ventral to portion of anterior testis nearest mid-line of body, and anterior to intestinal bifurcation. Metraterm thick-walled, ventral to cirrus pouch, opening into genital atrium. Uterus (opposite anterior testis) and metraterm amphitypic, ascending from right if anterior testis on left or vice versa. Eggs large, thick-shelled, operculate, containing miracidia. Vitelline follicles lateral, beginning at level of posterior testis or entirely posttesticular, ventral to ceca, terminating postcecally at level of ovary. Vitelline duct arises from each viltelline field, joining mid-ventrally to form short common vitelline duct.

Excretory vesicle with postero-dorsal opening. Lymph system present.

Mean measurements in millimeters (with minima and maxima in parentheses) of 10 whole mount adults from *Synodontis schall* are: body, length 2.005 (1.544-2.580), width 0.596 (0.416-0.739); oral sucker, 0.256 (0.200-0.330) x 0.200 (0.139-0.271); pharyngeal diverticulae, 0.076 (0.056-0.090) x 0.103 (0.090-0.114); esophagus, length 0.474 (0.403-0.574); esophageal bulb, 0.122 (0.099-0.165) x 0.083 (0.070-0.092); acetabulum, width 0.975 (0.792-1.162); anterior testis, 0.327 (0.231-0.389) x 0.298 (0.231-0.350); posterior testis, 0.286 (0.231-0.370) x 0.309 (0.231-0.416); cirrus pouch, length 0.114 (0.105-0.135); ovary, length (from 1 sagittally sectioned worm) 0.185, width (from 1 cross sectioned worm) 0.119; older intrauterine eggs, 0.167 (0.158-0.178) x 0.097 (0.093-0.099); anterior end to genital pore, 0.359 (0.290-0.448); anterior end to anterior testis, 0.415 (0.363-0.541); anterior end to posterior testis, 0.954 (0.760-1.208).

Measurements of 1 whole mount adult from *Mormyrus kannume* are: body, 1.597 x 0.409; oral sucker, 0.198 x 0.145; pharyngeal diverticulae, 0.063 x 0.080; esophagus, length 0.330; acetabulum, width 0.700; anterior testis, diameter 0.211; posterior testis, diameter 0.198; anterior end to genital pore, 0.297; anterior end to anterior testis, 0.356; anterior end to posterior testis, 0.719.

HOSTS: *Synodontis schall* and *Mormyrus kannume,* freshwater fishes (families Synodontidae and Mormyridae, respectively).

HABITAT: Small intestine.

LOCALITY: Giza Fish Market, Giza Province, Egypt.

TYPE: U. S. Nat. Mus. Helm. Coll., No. 38291 (1 whole mount slide of type and paratype from *S. schall,* 3 slides of 1 worm from *S. schall* in serial sagittal section and 3 slides of another in serial cross section), and No. 38292 (1 whole mount slide of paratype from *Mormyrus kannume*).

The generic name (Gr. *basis,* pedestal + *diskos,* disc) refers to the pedestal-like form of the acetabulum. The specific name (Gr. *ectos,* outside + *orchis,* testis) refers to the extracecal position of the anterior testis.

Specimens from 3 *S. schall* were collected on September 6 and 13, 1952, and August 23, 1953, respectively, in mixed infection with *Sandonia* described below. The one adult from *M. kannume* was taken on September 20, 1952.

Of the 10 *B. ectorchis* measured from *S. schall,* 7 had the anterior testis on the right and 3 on the left, indicating that amphitypy is of frequent occurrence. The anterior testis was on the left in the worm from *M. kannume.*

Yamaguti (1953) lists 16 genera of Paramphistomidae from fish hosts. McClelland (1957) added the genera *Sandonia* and *Brevicaecum. Basidiodiscus* differs from all these genera in having its terminal acetabulum in the form of a pedestal-like appendage much wider than the base of the body. In having one testis extracecal and the other intercecal, *Basidiodiscus* further differs from all genera from fish hosts with the exception of *Sandonia.*

Sandonia sudanensis McClelland, 1957

DIAGNOSIS: Body oblong-lanceolate. Oral sucker terminal, mouth tilted slightly ventral. No oral sucker papillae, but some around anterior end of body at level of oral sucker. Pharyngeal diverticulae 2, saccular, thick-walled, dorso-lateral to esophagus. Esophagus short, terminating in large muscular bulb at intestinal bifurcation; latter at level of anterior margin or portion of anterior testis. Ceca simple, dorsal terminating at level of ovary near posterior extremity. Acetabulum a ventroterminal, muscular, puckerable cup. Pigment granules of disintegrated cercarial eyespots distributed in all directions from around oral sucker to ovary.

Testes 2, smooth, round, oblique, frequently in contact, occasionally overlapping, amphitypic. Anterior testis at anterior two-fifths of body length, intercecal but frequently extending anteriorly to overlap or project slightly beyond cecum at intestinal bifurcation. Posterior testis at middle of body length, intercecal but frequently overlapping cecum laterally or small portion projecting slightly extracecally. Vas efferens of posterior testis arises from antero-dorsal margin, ascending to join vas efferens coming from dorsal margin of anterior testis. Vas deferens inflated into seminal visicle before entering cirrus pouch. Cirrus winding, opening into extremely shallow genital

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All figures were drawn with the aid of a microprojector. The value of the scale is 0.2 mm for Figures 1, 2, 6-8, and 0.1 mm for Figures 3-5.

A, acetabulum; C, cirrus; CP, cirrus pouch; CV, common vitelline duct; E, egg; EB, esophageal bulb; ES, esophagus: GA, genital atrium; GP, genital pore; IC, intestinal cecum; LC, Laurer's canal; M, metraterm; MG, Mehlis' gland; O, ovary; OD, oviduct; OS, oral sucker; P, papilliform projection; SV, seminal vesicle; TA, anterior testis; U, uterus; VD, vas deferens; V, vitellaria.

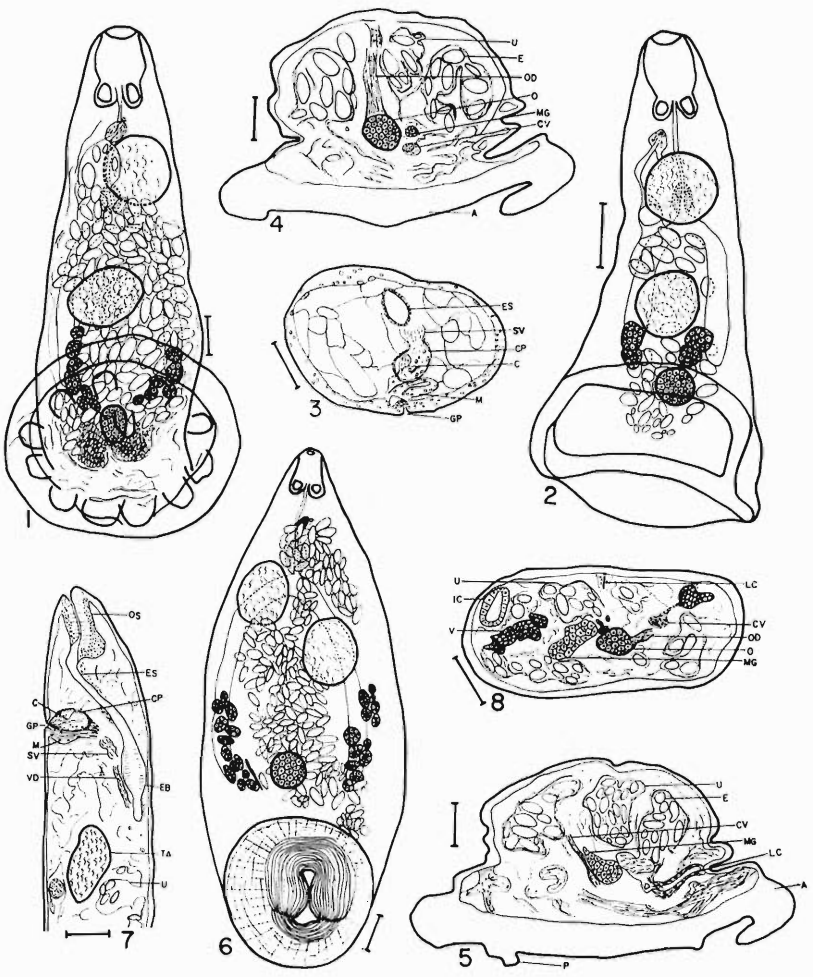

Figure 1. *Basidiodiscus ectorchis,* adult worm from *Synodontis schall,* ventral view. Acetabulum completely out of position due to flattening and mounting; should appear as a pedestal to which body is attached.

Figure 2. *Basidiodiscus ectorchis,* adult worm from *Mormyrus kannume,* ventral view. Acetabulum much distorted; should be as described for Figure 1.

Figure 3. Cross section of *B. ectorchis* from *S. schall* through cirrus pouch and metraterm.

Figure 4. Cross section of *B. ectorchis* from *S. schall* through ovary, oviduct, Mehlis; gland, and common vitelline duct. Acetabulum should not be in section, but is present due to its displacement from its normal position and being flattened against the body.

Figure 5. Cross section of *B. ectorchis* from *S. schall* through Mehlis' gland, common vitelline duct, beginning of uterus, and Laurer's canal. As for Figure 4 the acetabulum should not be in section.

Figure 6. *Sandonia sudanensis* McClelland. 1957, adult worm, ventral view.

Figure 7. Sagittal section of *S. sudanensis* through cirrus pouch. metraterm, esophageal bulb, and anterior testis.

Figure 8. Cross section of *S. sudanensis* through ovary, oviduct, Mehlis' gland, and Laurer's canal.

atrium. Vas deferens, seminal vesicle, and cirrus pouch amphitypic, ascending from right if anterior testis on left or vice versa. Genital pore mid-ventral, under or immediately anterior to esophageal bulb, without genital sucker.

Ovary central to mid-ventral, posttesticular, between cecal ends and postcecal near posterior end of body, elongate, smaller than either testis. Oviduct arises from postero-dorsal margin, passing dorsally into ootype; oviduct expands into uterus a short distance beyond ootype. Ootype complex contains Mehlis' gland, common vitelline duct, and Laurer's canal opening on dorsal surface. Uterine coils inter-, extra-, and postcecal to end of body, ventral and/or dorsal to testes. Metraterm thick-walled, opening into genital atrium immediately posterior to cirrus. Uterus (opposite anterior testis) and metraterm amphitypic, ascending from right if anterior testis on left or vice versa. Eggs large, thin-shelled, operculate, containing miracidia, some hatched within uterus of preserved specimens. Vitelline follicles lateral, beginning at level of posterior testis, ventral to ceca, terminating postcecally near posterior extremity of body. Vitelline duct arises from each vitelline field, joining medially to from short common vitelline duct.

Excretory vesicle with postero-dorsal opening. Lymph system present.

Mean measurements in millimeters (with minima and maxima in parentheses) of 9 whole mount adults are: body, length 2.274 (1.630-3.082), width 0.945 (0.746-1.056); oral sucker, 0.233 (0.172-0.297) x 0.159 (0.112-0.191); pharyngeal diverticulae, 0.108 (0.083-0.138) x 0.126 (0.090-0.170); esophagus, length 0.455 (0.260-0.660); esophageal bulb, 0.177 (0.158-0.198) x 0.135 (0.132-0.139); acetabulum, 0.667 (0.541-0.884) x 0.678 (0.541-0.752); anterior testis, 0.356 (0.264-0.449) x 0.361 (0.264-0.475); posterior testis, 0.339 (0.297-0.429) x 0.336 (0.304-0.442); ovary, length (from 3 worms) 0.186 (0.162-0.211), width (from 4 worms) 0.169 (0.144-0.224); older intrauterine eggs, 0.156 (0.145-0.165) x 0.091 (0.086-0.099); anterior end to genital pore, 0.472 (0.343-0.653); anterior end to anterior testis, 0.675 (0.475-1.023); anterior end to posterior testis, 0.916 (0.627-1.399).

HOST: *Synodontis schall*, a freshwater siluroid fish (family Synodontidae).

HABITAT: Small intestine.

LOCALITYS Giza Fish Market, Giza Province, Egypt.

SPECIMENS: U. S. Nat. Mus. Helm. Coll., No. 38293 (3 whole mount slides, 3 slides of 1 worm in serial sagittal section, and 3 slides of 1 worm in serial cross section).

Specimens from 3 *S. schall* were collected on September 6 and 13, 1952, and August 23, 1953, respectively, in mixed infection with *Basidiodiscus ectorchis*. Additionally 2 hosts taken August 16 and September 20, 1952, respectively, harbored *S. sudanensis* alone.

Amphitypy occurs frequently in this species as 5 had the anterior testis on the right and 4 on the left.

A detailed description of *S. sudanensis* has been presented in order to amplify the description given by McClelland (1957) and to point out several differences. Inasmuch as McClelland measured both smaller and larger worms, most of his measurements have a wider range than those for this paper. The vas deferens of worms in the present study is essentially a straight duct inflating into a seminal vesicle prior to entering the cirrus pouch, whereas in McClelland's specimens the vas deferens is very greatly coiled and the seminal vesicle is described as within the cirrus pouch.

LITERATURE CITED

McCLELLAND, W. F. J. 1957. Two new genera of amphistomes from Sudanese freshwater fishes. Jour. Helminth. 31: 247-256.

YAMAGUTI, S. 1953. Systema Helminthum. Part I. Digenetic Trematodes of Fishes. 405 pp. Maruzen Co., Tokyo.

Survival on Pasture of Larvae of Gastrointestinal Nematodes of Cattle. II. Spring Contamination

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This paper is the second of a series reporting the outcome of tests to obtain data on the development and survival on pastures of the free-living stages of nematodes of cattle under the climatic conditions prevailing at the Agricultural Research Center, near Beltsville, Maryland. In the previous paper, Goldberg and Rubin (1956) reported the results obtained following the contamination of pasture plots with feces in midsummer. The results obtained following the contamination of plots in the spring are given in the present paper.

MATERIALS AND METHODS

A nearly level portion of a permanent pasture was divided into five 2,040-sq. ft. rectangular plots (plots 1-5). The ground was well covered by vegetation consisting of about 95 per cent Kentucky bluegrass and 5 per cent various other herbs (about 25 species). The plots were in full sun. A boundary fence was erected around each; each was ditched around its borders to prevent contaminated material from being washed from it or into it.

On May 4, 1956, an equal, weighed aliquot of a 24-hr. collection of feces from 13 infected cattle was distributed fairly evenly over a 4-ft. wide, crosswise strip on each of the plots. This process was repeated daily, contaminating a new strip across each plot each day, until distribution of the manure over the last strip was completed on May 28, 1956.

On May 3 and May 28, and at 6-day intervals between these dates, total and differential counts of the nematode eggs per gram of a composite sample of fresh feces from the aforementioned cattle were made using a direct centrifugal flotation method. From these counts and the weight of feces placed on each plot in each period, the total number of eggs and the number of each genus distributed over each plot were computed.

Except for very limited examinations of herbage and fecal samples by means of the Baermann apparatus, data on the development and survival of infective eggs and larvae on the plots were obtained by means of grazing tests as described by Goldberg and Rubin (1956). Except as noted below, all other procedures and methods employed, including those used to obtain the meteorological data, also were similar to those described by these authors.

Five test calves were used, one for each plot. Their ages ranged from 3.1 to 6.7 months when put out to graze. The intervals from the end of the contaminating period to the test grazing of plots 1-5 are shown in tables 2 and 3. Plots 2-5 were mown on July 2, 1956; the mowings were left as they fell. The grazing period on plots 3-5 was extended 3 or 4 days longer than 2 weeks to insure that each plot was grazed very closely and that all were grazed equally closely. However, no calf was left on a plot long enough

for autoinfection to occur. The feces of each were examined, and egg counts were made on the 15th day after the calf was put out to graze, and at three, approximately weekly, intervals thereafter; if eggs were present on the first examination, the calf was removed from the plot within 2 days.

To estimate the number of *Oesophagostomum* larvae in intestinal lesions at autopsy, the number of larvae obtained by dissection of 20 of the nodules was used; if fewer nodules were present, all were dissected.

METEOROLOGICAL DATA

From the beginning of the contaminating period to the beginning of the first grazing test, precipitation was 2.7 in. and the mean temperature was 4.7° F. below average for the vicinity (table 1). In the fifth test period, scarcely more than a trace of precipitation fell and the mean temperature was considerably above average (+7.6° F.). Marked deviation from normal precipitation also occurred during the second grazing test, when only about one-tenth the normal amount fell. During the entire period of the experiment, precipitation was about 20 per cent below normal. Other periods in which the mean temperature deviated more than 3° F. from the average were during the third test (+3.2° F.), between the third and fourth tests (+5.1° F.), and during the fourth test (−4.9° F.).

There was dew on the forage on several days during the first test calf's stay on pasture. During the fourth test, light frost formed on several nights and heavy frost on one night.

RESULTS

EGGS PER PLOT. The estimated number of nematode eggs contained in the feces on each plot was 94,745,000; approximately 50, 21, 21, 6, 2, 0.06, and 0.04 per cent of them were identified as *Cooperia, Ostertagia, Trichostrongylus, Oesophagostomum, Haemonchus, Nematodirus,* and *Trichuris,* respectively. However, the method of fecal examination used floated *Trichuris* eggs less effectively than eggs of the other genera named.

DEVELOPMENT AND ACCESSIBILITY OF INFECTIVE LARVAE. The following numbers of infective larvae were recovered from random samples of the feces and the herbage on one of the plots on June 13, sixteen days after the end of the contaminating period: feces, 8.2 per gram, and upper and lower half of herbage, 9.6 and 9.1 per gram, respectively. On the basis of the weight of the herbage mown from a similar plot and the weight of the manure deposited per plot, with allowance for diminution in the weight of the feces due to weathering and other causes, these counts suggested that roughly 600,000 infective larvae were accessible on the herbage on this plot at this time and that there was a reserve of at least 2,500,000 in the feces. Most of the larvae recovered were identified as *Cooperia, Ostertagia,* and *Trichostrongylus;* about 20 per cent of those from the feces, 1 per cent of those from the lower half, and none of those from the upper half of the herbage were identified as *Oesophagostomum.*

Evidently, considerable numbers of infective larvae likewise were in a position for prompt ingestion 21 and 63 days after the end of the contaminating period since the feces of the calf put out to graze at each of these times were positive for nematode eggs 15 days later (table 2).

LARVAL SURVIVAL AS SHOWN BY WORMS RECOVERED. The number of worms, including *Oesophagostomum* larvae in nodules, recovered from the digestive tract of calf 1, placed on plot 1 twenty-one days after the contaminating

Table 1. Comparison of local weather data for each period during the experiment with corresponding 8-year averages for Washington, D. C.

Period	Dates	Temperature (degrees F.)						Precipitation (inches)	
		Average		Av. daily max.		Av. daily min.			
		Agr. Res. Center	Wash. D.C.	Agr. Res. Center	Wash. D.C.	Agr. Res. Center	Wash. D.C.	Agr. Res. Center	Wash. D.C.
Contamination to first test	5- 4 to 6-17-56	63.1	67.8	76.7	77.8	49.5	57.8	2.88	5.57
First test	6-18 to 7- 2-56	72.3	73.5	82.2	83.2	62.3	63.9	1.81	1.99
Interval between 1st and 2nd tests	7- 3 to 7-29-56	74.3	77.1	83.5	86.6	65.0	67.8	5.01	3.87
Second test	7-30 to 8-14-56	75.1	75.4	86.3	84.6	63.8	66.2	0.21	2.25
Interval between 2nd and 3rd tests	8-15 to 9-30-56	70.0	71.1	83.1	80.5	56.8	61.9	4.53	6.09
Third test	10- 1 to 10-18-56	60.7	57.5	73.4	67.3	48.0	47.8	1.08	1.72
Interval between 3rd and 4th tests	10-19 to 11-14-56	56.7	51.6	65.8	60.9	47.6	42.6	3.67	2.47
Fourth test	11-15 to 12- 3-56	39.8	44.7	52.3	53.4	27.2	36.4	1.55	1.70
Interval between 4th and 5th tests	12- 4 to 4-21-57	40.9	40.3	51.1	48.8	30.7	31.9	13.77	15.08
Fifth test	4-22 to 5-10-57	67.1	59.5	83.0	69.6	51.2	49.4	0.09	2.17

Table 2. Worm egg counts of calves after grazing on test plots 1-5.

Calf and plot No.	Grazing period		Number of eggs per gram of feces				
	Days from end of contamination to start	Dates	On indicated days after initial exposure				Average
			15th	21st	28th	35th	
1	21	6-18 to 7- 2-56	85	810	1,200	2,890	1,246
2	63	7-30 to 8-14-56	4.5	444	950	870	567
3	126	10- 1 to 10-18-56	0	65	615	1,135	454
4	171	11-15 to 12- 3-56	0	10	175	618	201
5	329	4-22 to 5-10-57	0	0.7	19	118	34

period ended, was estimated to be approximately 277,000. The recoveries from calves 2-5 put out subsequently to graze, as shown in table 3, were about 32, 8, 9, and 0.5 per cent of this number, respectively. Evidently, therefore, a sharp reduction in the population of infective larvae occurred in the first 6 weeks of summer between the first and second grazing tests. A further large reduction occurred during the next 9 weeks, extending into early fall; apparently there was no reduction in the next 7.5 weeks of the fall, but by the following April only a relatively small number of larvae were still viable.

The numbers of worms recovered from the calves which grazed plots 1-5 were 0.29, 0.094, 0.024, 0.026, and 0.0015 per cent, respectively, of the number of eggs deposited per plot.

OTHER INDICES OF SURVIVAL. Although the decline with time in the infectiousness of the plots was also reflected in the fecal egg counts (table 2) of the test calves, these counts were a much less accurate index of the survival of the infective larvae than were the worm recoveries.

The decline in infectiousness was also reflected by the clinical effects of the infections acquired by the several test calves. Calf 1 became very sick. It lost 52 lb. in the 3-week period after removal from the test plot. It developed a watery diarrhea, whereas none of the subsequent test calves became diarrheic. It was unable to stand on the day of autopsy. Calf 2 gained only 4 lb. in 1.2 months from the time it was placed on the test plot to the time of slaughter. No marked effect on weight gain was noted in test calves 3-5 in a similar period.

DEVELOPMENT AND SURVIVAL OF DIFFERENT GENERA AND SPECIES. The ratio of the maximum number of worms of each genus recovered from any test calf, usually calf 1, to the number of eggs of the same genus deposited per plot indicated that the percentage development of eggs into viable infective larvae was greatest for *Trichostrongylus* and *Nematodirus* and lowest for *Haemonchus*. *Cooperia punctata* larvae apparently were present on the herbage in peak numbers earlier than were *C. oncophora* larvae. Among the strongylids and trichostrongylids tested, larvae of *C. oncophora* and *Nematodirus* survived longest and those of *Ostertagia* survived next longest. Only larvae of the first two kinds and eggs of *Trichuris* survived over winter and for the 329-day period of the experiment; the limit of their ability to survive was not determined.

DISCUSSION

Since the ultimate purpose of studies of the survival of parasite larvae on pastures is to provide data for the formulation of pasture management practices for the control of parasitism, the writers, Goldberg and Rubin (*loc. cit.*), Kates (1950), and others have employed the method of test grazing instead of the method of direct isolation of the larvae from the substrate. Each method has its deficiencies for the measurement of survival. However, the latter method has the disadvantage that it affords no direct measurement of the actual infectiousness of the test area at any stage in the decline of the larval population, since the living larvae recovered are not necessarily infectious. Some of the deficiencies of the grazing method have been mentioned by Kates (*loc. cit.*); other factors that may be presumed to affect its accuracy are variability among test animals in general susceptibility, and in susceptibility to particular species, and differences in meteorological conditions during different grazing periods. Much remains to be learned about

Table 3. Kinds and numbers of worms recovered from, and number of *Oesophagostomum* lesions in, calves placed on test plots 1-5 at indicated intervals* after final date of contamination, May 28, 1956.

	21 Days (6-18 to 7-2-56)	63 Days (7-30 to 8-14-56)	126 Days (10-1 to 10-18-56)	171 days (11-15 to 12-3-56)	329 Days (4-22 to 5-10-57)
Ostertagia ostertagi	6,950	3,315	350	200	0
Trichostrongylus axei	157,250	1,500	0	0	0
*Haemonchus contortus***	0	32	0	0	0
Cooperia punctata	99,390	6,570	0	0	0
Cooperia oncophora	2,465	64,640	10,050	11,025	1,275
Cooperia larvae	7,000	11,750	11,850	13,575	0
Nematodirus helvetianus (including *Nematodirus* larvae)	14	670	310	225	8
Oesophagostomum radiatum (including larvae in nodules)	4,100	470	6	0	0
*Trichuris ovis***	***	27	24	60	160
Totals****	277,170	88,970	22,590	25,090	1,440
Oesophagostomum lesions	4,200	430	11	0	0

* Dates of grazing period in parentheses.
** *Sensu lato.*
*** Not examined for *Trichuris.*
**** To nearest 10.

the behavior of infective larvae, particularly as to their migrations on herbage in response to differences and changes in environmental conditions. It is, therefore, worth noting that larvae were present on the herbage at the time of the final grazing test of the present experiment, although almost no rain fell during this 2-week period.

Comparison of the results obtained by the writers and by Goldberg and Rubin (*loc. cit.*) indicates that a greater percentage of eggs developed into infective larvae and that larval survival, at least up to 4 months, was greater on a pasture contaminated in spring than on a comparable one contaminated in summer; the indices compared were the ratio of the number of worms recovered from the first test calf to the number of eggs deposited per plot and the ratio of this number of worms to the numbers recovered from the calves put out subsequently to graze. In both experiments the pasture was much less infectious 2 months after the end of the contaminating period than it was 3 weeks after this period, but infectiousness was not reduced as much in about 6 months after spring contamination as in 2 months after summer contamination (about 92 versus about 98 per cent).

Ostertagia larvae survived over winter in the summer contamination experiment, but not in the spring contamination experiment; however, the total duration of exposure to the environment was only about 8.5 months in the former experiment, whereas it was about 11 months in the latter one. *Nematodirus* and *C. oncophora* larvae and *Trichuris* eggs survived over winter in both experiments.

SUMMARY

In the spring of 1956, each of five small pasture plots at Beltsville, Maryland, was contaminated with cattle feces estimated to contain about 95,000,000 eggs of common nematode parasites of cattle. Each plot was later grazed closely by a different, initially worm-free calf. The numbers of worms recovered from test calves were used as the main index of the decline in infectiousness of the plots. The calves put out to graze 2, 4, and 11 months, respectively, after the end of the contaminating period yielded 68, 92, and 99.5 per cent fewer worms, respectively, than did the first calf put out to graze 3 weeks after the end of the contaminating period. There was no decline in the infectiousness of the plots between the fourth and sixth months postcontamination.

Infective larvae of *Cooperia oncophora, Nematodirus helvetianus,* and *Ostertagia ostertagi* survived longer than those of *Trichostrongylus axei, Cooperia punctata,* and *Oesophagostomum radiatum.* Some larvae of the first two of these species and *Trichuris* eggs survived over winter. *Haemonchus* larvae developed only in small numbers in this experiment.

A deficiency in precipitation was the main deviation from normal meteorological conditions.

The percentage of infective larvae which survived for 2 months was considerably higher, and the percentage which survived for 4 months was somewhat higher in this spring contamination experiment than in a previously reported summer contamination experiment carried out at Beltsville.

LITERATURE CITED

GOLDBERG, AARON, and RUBIN, ROBERT. 1956. Survival on pasture of larvae of gastrointestinal nematodes of cattle. Proc. Helminth. Soc. Wash. 23: 65-68.

KATES, KENNETH C. 1950. Survival on pasture of free-living stages of some common gastrointestinal nematodes of sheep. Proc. Helminth. Soc. Wash. 17: 39-58.

The Infective Larva of *Nematodirella longispiculata antilocaprae* (Nematoda: Trichostrongylidae)

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Nematodirella longispiculata antilocaprae (Price, 1927) Dikmans, 1935,† is a very common roundworm of the small intestine of the prong-horned antelope (*Antilocapra americana*). In several Western States, this wild ruminant abounds on many ranges used for cattle and sheep. In Wyoming, at least, some summer ranges are frequented also by moose, of which *N. l. alcidis* Dikmans, 1935,‡ is a common parasite. As a result of the presence of these wild ruminants, forage and soil samples, collected from such ranges to investigate the effect of prevailing conditions on the development, availability, or survival of the larvae of the several genera of nematodes that normally occur in cattle and sheep, are very likely to be contaminated with infective *Nematodirella* larvae. That the degree of contamination can be rather heavy is suggested by the fact that sheep, though not a normal host, sometimes acquire *Nematodirella* infections in Wyoming (Honess, 1951).

Since an ecologic investigation of this type requires indentification and differential enumeration of all larvae isolated from the samples collected, the question of whether it is possible to distinguish *Nematodirella* larvae from the larvae of the cattle and sheep nematodes is of considerable importance; if this distinction is impossible, the fact must be taken into account in evaluating and reporting the results obtained.

To answer this question satisfactorily, a description of an infective-stage larva of this genus is needed; since none has been found in the available literature, the writers have cooperated in the present effort to fill this need. One of us (R.F.H.), whose experiences in carrying out ecologic investigations of parasites of cattle on range lands in Wyoming supplied the impetus for this undertaking, obtained the small intestine of a freshly killed prong-horned antelope and collected from it several living female worms, which he identified as *N. l. antilocaprae*. He dissected out their eggs and shipped these in a water culture to Beltsville, Maryland, where the structure of the infective larvae that issued from these eggs was determined and all other original observations included in this report were made; both heat-killed and iodine-killed specimens were examined.

DESCRIPTION: Larva large, enclosed in a sheath with a very long attenuated tail process; principal size relationships of 10 specimens as given in table 1. Definite lips not evident; a pair of amphids, and apparently two pairs of submedian cephalic papillae, present. Oral opening minute, communicating with stoma proper (buccal cavity) via a fine canal (cheilostom ?) about 3 microns long. Buccal cavity (Fig. 1, A) essentially cylindrical, about 8 to 13 microns long, its wall in median optical section appearing as two discrete pairs of sclerotized rods, a relatively short, anterior pair (prorhabdions ?) and a relatively long, posterior pair (metarhabdions ?); in killed specimens, the shorter pair may converge anteriorly and the longer pair may converge posteriorly, the buccal cavity appearing diamond-shaped; in living specimens, the rods are nearly parallel to the body axis. Esophagus more or less cylindrical, not highly muscular, without a pronounced terminal post-

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†Synonyms: *Nematodirus antilocaprae* Price, 1927: *Nematodirella antilocaprae* (price, 1927) Ivaschkin, 1954.
‡Synonym: *Nematodirella alcidis* (Dikmans, 1935) Ivaschkin, 1954.

corpal bulb; surrounded by numerous large nerve cells, obscuring observation of details of its structure. Ratio between (1) distance from cephalic tip of larva to excretory pore (116-145 microns; av. 133) and (2) distance from cephalic tip to base of esophagus, approximately 2:3. Nerve ring about 8 to 25 microns anterior to excretory pore. Width of larva plus sheath at posterior end of esophagus 26-34 microns. Intestine (Fig. 1, C) composed of 8 large, uninucleate cells, appearing to be roughly trapezoidal in outline and to be arranged in a single row or chain (tandem) when larva is viewed exactly laterally. Genital primordium oval, about 22 to 25 microns long, situated in a concavity of the ventral surface of the fifth intestinal cell; a triangular genital giant cell, adjacent to anterior end of primordium, observed in most specimens. Tail of larva proper (Fig. 1, B) terminating in 4 processes: (1) a more or less medial, fingerlike one, about 10 to 18 microns long; (2) a dorsal, bluntly conical one, usually about 5 to 8 microns long, but sometimes somewhat shorter; (3) a pair of minute, ventrolateral, sharply conical ones, about 2 to 5 microns long. Sheath thickest in region normally enclosing tip of tail of larva; pronounced tapering of sheath to its fine, but not sharply pointed, posterior tip, beginning in this same region; lumen sometimes extending as much as 100 microns posteriorly beyond tip of larval tail.

REMARKS: Various authors, notably Dikmans and Andrews (1933), Andrews (1935), and Keith (1953), have provided descriptions of infective larvae of each of the several genera of gastrointestinal nematodes that commonly occur in domestic ruminants and have shown how they may be distinguished from one another. The same combination of characteristics that has been shown to differentiate larvae of typical *Nematodirus* from larvae of the rest of these genera, *i.e.*, larger size and greater length of tail of sheath, 8-celled intestine, and the trifid appearance of the tip of the tail of the larva proper, serves for the differentiation of the larva of *Nematodirella l. antilocaprae* from larvae of these same genera. Accordingly, the possibility of differentiating this larva from *Nematodirus* larvae depends upon whether it differs from them in (1) size, or size relationships, (2) arrangement of the intestinal cells, (3) number of processes at the tip of the tail of the larva proper, or (4) some other minor respect.

A comparison of the measurements given in table 1 with the corresponding measurements reported for larvae of two of the most common species of *Nematodirus, N. spathiger* and *N. helvetianus,* as summarized by Herlich (1954), has shown that differentiation between these three species cannot reliably be made on the basis of size, since there is overlapping in the ranges of all indices that have potential value.

Table 1. Principal measurements in microns of 10 infective-stage larvae of *Nematodirella longispiculata antilocaprae.*

	Range	Mean	Standard Deviation
Total length including sheath	964–1083	1024	43
Length of larva proper	667– 786	740	35
Length of esophagus	170– 206	189	13
Distance from anterior end to mid-point of genital primordium	369– 446	407	25
Length of tail of larva proper	65– 83	70.5	5.6
Distance from anus to posterior tip of sheath	303–382	354	27
Distance from tip of larval tail to posterior tip of sheath	234–319	284	28

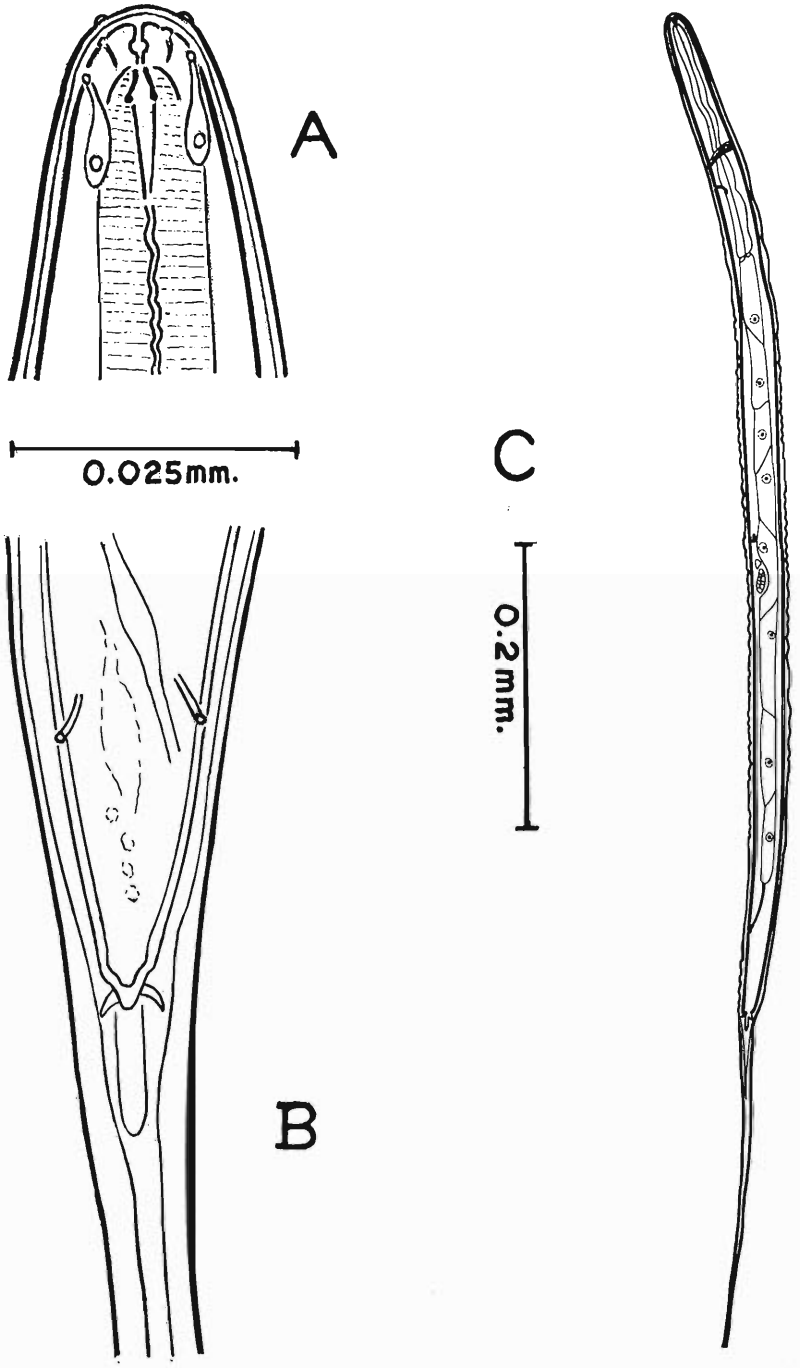

Fig. 1. *Nematodirella l. antilocaprae*, infective-stage larva: A, anterior region, dorsoventral aspect; B, tail region, showing four terminal processes, dorsoventral aspect; C, entire larva, lateral aspect.

Dikmans and Andrews stated that in *N. spathiger* larvae, the intestine consists of 8 cells arranged alternately, and illustrated them (1933, Pl. II, Fig. 3) as forming two rows, one dorsal and one ventral, of more or less opposite cells. Thomas (1957) reported this same arrangement of the intestinal cells in larvae of this species, *N. filicollis*, and *N. battus*. However, a tandem arrangement of most of the cells is rather clearly indicated in Kates and Turner's photomicrograph (1955, Pl. 1, Fig. 7) of a larva of *N. spathiger*, and on examination of specimens for the purpose of the present report it was found that all, or all but the anteriormost two, of the cells are usually so arranged in larvae of this species oriented exactly laterally. Herlich stated that the larva of *N. helvetianus* has 8 alternate intestinal cells—4 dorsal and 4 ventral, but his illustration (1954, Pl. 1, Fig. G) of this larva shows them as arranged in a single chain in lateral view. Hence, it appears reasonably evident that, despite these descriptive discrepancies, the structure of the intestine is alike in larvae of at least some species of *Nematodirus* and the larva here described. The manner in which the cells appear to be arranged undoubtedly depends upon the orientation of the larva and the factor of torsion in the intestine.

The tip of the tail of the larva of *N. spathiger* was described by Dikmans and Andrews as "forked," with a rod-shaped process situated between "the dorsal and ventral projections"; their illustration (1933, Pl. VI, Fig. 4) shows one large and two small terminal processes. Herlich (1954) gave a similar description of the termination of the tail in the larva of *N. helvetianus*. However, examination of several specimens for the purpose of including the findings in the present report has shown that actually, as is the case in the larva here described, the tail of the larva of *N. spathiger* ends in 4 processes, two ventrolateral ones (rather than a single ventral one) being present. Thomas (1957) found that in *N. filicollis* the larval tail, though tricuspid terminally, lacks a median rodlike process and that in *N. battus* the tail tapers to a fine point and bears two notches dorsally.

The buccal cavity has been illustrated (Andrews, 1935) as somewhat globular anteriorly and tubular posteriorly in the larva of *N. spathiger*, and Herlich (1954) described it as hexagonous in the larva of *N. helvetianus* and apparently considered this to be a specific difference. In the larva here described, it usually has an elongate-diamond shape in heat-killed specimens. Experience has shown, however, that the buccal cavity may show all of these differences in shape in killed larvae of a single species, its exact shape being dependent on the technique employed and other extrinsic factors.

Therefore, there appears to be no dependable means available for the distinction of the larva of *Nematodirella l. antilocaprae* from larvae of some *Nematodirus* species. This is further evidence of the close relationship (Douvres and Lucker, 1958) between the genera *Nematodirus* and *Nematodirella*.

BIBLIOGRAPHY

ANDREWS, JOHN S. 1935. A note on the morphology of the anterior ends of the infective larvae of some nematodes parasitic in the alimentary tract of sheep. Proc. Helminth. Soc. Wash., 2:88-90.

DIKMANS, G. and ANDREWS, JOHN S. 1933. A comparative morphological study of the infective larvae of the common nematodes parasitic in the alimentary tract of sheep. Trans. Amer. Micr. Soc., 52:1-25.

DOUVRES, FRANK W. and LUCKER, JOHN T. 1958. A note on the genera *Nematodirus* Ransom, 1907, and *Nematodirella* Yorke and Maplestone, 1926 (Nematoda: Trichostrongylidae). Proc. Helminth. Soc. Wash., 25:48-52.

HERLICH, HARRY. 1954. The life history of *Nematodirus helvetianus* May, 1920, a nematode parasitic in cattle. Jour. Parasitol., 40:60-70.
HONESS, RALPH F. 1951. Common helminths of Wyoming sheep. Bull. (305) Wyoming Agric. Exper. Sta., 24 pp.
KATES, K. C. and TURNER, J. H. 1955. Observations on the life cycle of *Nematodirus spathiger*, a nematode parasitic in the intestine of sheep and other ruminants. Amer. Jour. Vet. Res., 16:105-115.
KEITH, R. K. 1953. The differentiation of the infective larvae of some common nematode parasites of cattle. Austral. Jour. Zool., 1:223-235.
THOMAS, R. J. 1957. A comparative study of the infective larvae of *Nematodirus* species parasitic in sheep. Parasitology, 47:60-65.

Case Reports of the Quill Mite, *Syringophilus bipetcinatus*, in Poultry

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Two young White Leghorn chickens, which were raised on a farm near Bowie, Maryland, and suspected of being infected with intestinal capillarid worms, were recently presented to the writer by Dr. H. M. DeVolt of the Livestock Sanitary Service of the University of Maryland. When these birds were examined externally, a number of flight feathers had been broken off and others could easily be pulled out or loosened. The interior of the quills of these feathers was filled with a brownish powder instead of the normal white pith (fig. 1). This brownish powder, when examined under the microscope, contained, along with a great deal of debris, a large number of mites, both young and adult. These mites were identified as *Syringophilus bipectinatus* Heller, 1880. Subsequently, the writer also received two lots of feathers infested with quill mites from Dr. L. Leibovitz of the Poultry Diagnostic Laboratory of Doylestown, Pennsylvania. A comparison of these materials with those of the chickens from Bowie, Maryland, showed that they all belonged to the same species.

The body of the adult of this mite is elongated, having the characteristic M-shaped peritreme in the region of the gnathosoma and the long setae at the posterior end. The average body length is 0.8 mm. in the female and 0.62 mm. in the male; the average body width is 0.18 mm. in the female and 0.17 mm. in the male (figs. 2 and 3). The ova are approximately 0.26 mm. long and 0.15 mm. wide.

S. bipectinatus was first described by Heller in 1880 as a parasite of chickens and pigeons. This mite was next reported to be found on feathers of the black flycatcher, *Phoenopepla nitus*, in the United States by Hancock in 1895. Rebrassier and Martin (1932) reported it in five widely separated flocks of chickens in Ohio in 1930. These authors stated that in about 75 per cent of a flock of 1,500 birds, some of which were known to be infested with the quill mite, a "peculiar molt," or loss of feathers, had been noticed for nearly a year. Schwabe (1956) reported that during the year of 1955, seven cases of flock infestation by the quill mite, *S. bipectinatus*, were recognized at the New Jersey State Poultry Laboratory in Vineland. This author stated that of the 29 birds examined, 22, or roughly 76 per cent, were infested. However, a much higher incidence than that indicated by these few published observations might be revealed were birds more carefully examined for these parasites at the time of autopsy. The effect of an infestation with

this mite on egg production and the general health of the chickens has not been evaluated, but the presumption is that beyond the loss of feathers and the unsightly appearance of the birds, very little damage results.

S. columbae, a species closely related to *S. bipectinatus*, is reported to frequently infest pigeons in the United States. Since both species have been reported from pigeons in other countries, further investigation may show that *S. bipectinatus* likewise occurs on this host in the United States.

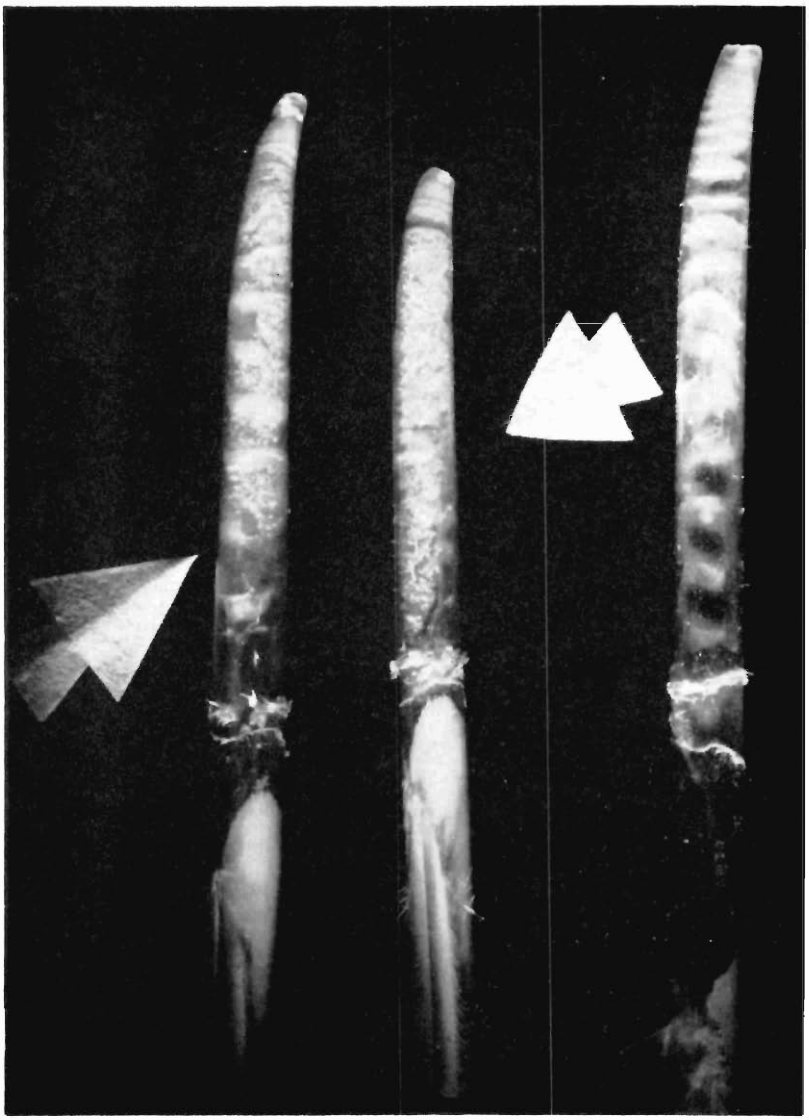

Figure 1. A comparison of the healthy quill of a flight feather of a chicken (left) with quills infestated with *Syringophilus bipectinatus* mites (center and right).

According to Wilson (1958), *S. bipectinatus* is readily differentiated from *S. columbae* in that (1) the former has 8-12 cells in the longitudinal arm of the M-shaped peritreme, whereas the latter usuallly has only 3-4 cells, which are much longer than broad; and (2) the former has three pairs of setae, with the two anterior pairs situated close together, on the dorsal surface of the propodosoma, whereas the latter has only two pairs of setae—one pair situated anteriorly and one pair posteriorly.

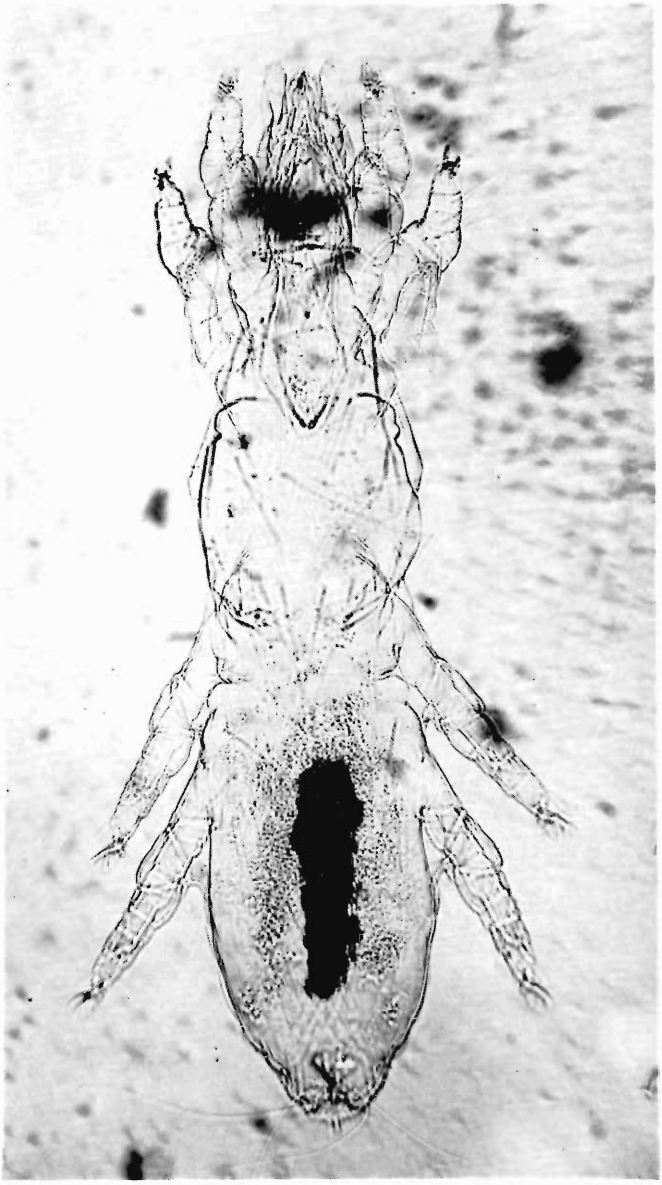

Figure 2. An adult female *Syringophilus bipectinatus*.

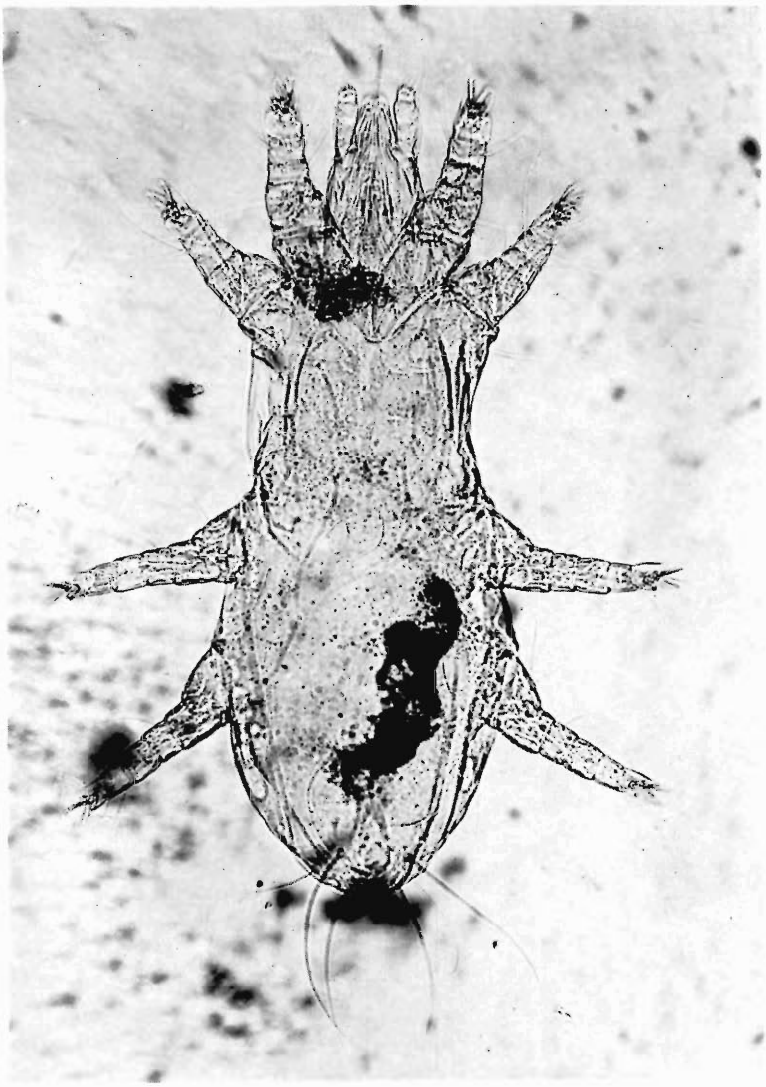

Figure 3. An adult male *Syringophilus bipectinatus*.

LITERATURE CITED

HANCOCK, J. L. 1895a. A new trombidian. Amer. Naturalist 29: 382-384.

———. 1895b. *Picobia villosa* (Hancock). A response to E. L. Trouessart. Amer. Naturalist 29: 866-867.

HELLER, A. 1880. Die Federspülmilben die Schmärotzer. Leipzig, pp. 186-188. (Original not seen; unavailable.)

REBRASSIER, R. E., and MARTIN. E. D. 1932. *Syringophilus bi-pectinatus*, a quill mite of poultry. Science 76: 128.

SCHWABE, O. 1956. A quill mite of poultry—a case report. J. Amer. Vet. Med. Assoc. 129: 481-482.

WILSON, G. 1958. Personal communication.

The Male of *Scutellonema brachyurum* (Steiner, 1938) Andrassy, 1958*

DONALD P. TAYLOR**

In 1958 Andrassy (1958) established the genus *Scutellonema* into which were placed those species of *Rotylenchus* Filipjev, 1934, having large phasmids (scutella). Of these, males have been described only of *S. christiei* (Golden and A. L. Taylor, 1956) Andrassy, 1958, *S. bradys* (Steiner and LeHew, 1933) Andrassy, 1958, and *S. blaberum* (Steiner, 1937) Andrassy, 1958. The original authors did not describe males of *S. brachyurum* (Steiner, 1938) Andrassy, 1958, *S. coheni* (J. B. Goodey, 1952) Andrassy, 1958, and *S. boocki* (Lordello, 1957) Andrassy, 1958.

In 1957, a diseased specimen of African violet (*Saintpaulia ionantha* Wendl.) from St. Joseph, Minnesota, was discovered to be parasitized by a large population of *Scutellonema brachyurum,* a known endoparasite of this plant (Golden, 1956). This population has been increased and maintained on African violets in the greenhouse since that time.

Recent examination of these cultures revealed that males of this species occurred in the ratio of about one to every 200 females. This paper presents the first description of males of *S. brachyurum.*

Scutellonema brachyurum (Steiner, 1938) Andrassq, 1958. Males:

VALUES: 10 Males: L = .577 ± .042 mm. (.531-.645 mm.)†; a = 28.1 ± 2.4 (23.2-30.8); b = 5.6 ± 0.6 (5.1-6.8)ffi c = 45.2 ± 3.4 (40.8-50.2); Stylet = 25.4 ± 0.3 μ (23.8-26.8 μ).

DESCRIPTION: Body, assuming open spiral when relaxed, resembles that of female although considerably shorter and more slender.

Anterior end morphologically similar to female. Three or four striae on lip region. Stylet shorter and more slender than in female, with basal knobs slightly flattened anteriorly. Excretory pore located opposite junction of esophagus and intestine. No deirids observed.

Testis single, outstretched, approximately one-half body length. Cells arranged as shown in Figure 1A. Sperm spherical, approximately 2μ in diameter. Spicula averaging 24μ in length. Gubernaculum about 1/3 spicule length. Conspicuous bursa envelopes tail. Phasmids (scutella) large, opposed, located at level of or immediately posterior to cloacal opening.

ALLOTYPE: Slide H-1: Minnesota Nematode Collection, St. Paul, Minnesota.

DIAGNOSIS: Males of *S. brachyurum* can be distinguished from males of *S. christiei* by the presence of four lines in the lateral field and by the presence of opposed scutella on the tail. From males of *S. bradys* and *S. blaberum,* males of *S. brachyurum* can easily be recognized by the presence of only three or four striae on the lip region.

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†The first figure for each dimension is the mean of the population, while the second figure is the standard deviation from the mean. The observed range is included in parentheses.

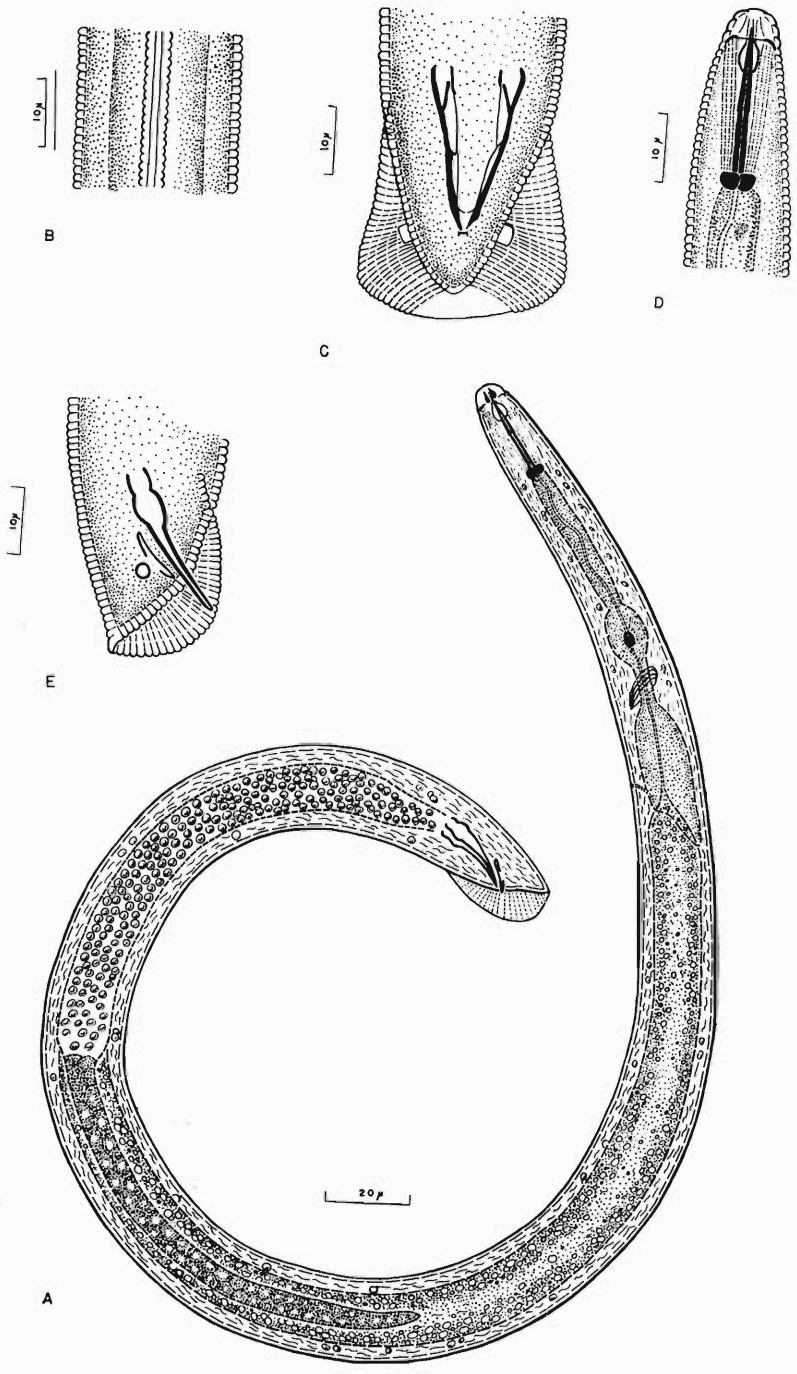

Figure 1. *Scutellonema brachyurum* male. A. Adult male. B. Lateral field. C. Ventral view of tail. D. Anterior end. E. Lateral view of tail.

LITERATURE CITED

ANDRASSY, I. 1958. *Hoplolaimus tylenchiformis* Daday, 1905. (Syn. *H. coronatus* Cobb, 1923) und die Gattungen der Unterfamilie Hoplolaiminae Filipjev, 1936. Nematologica 3:44-56.

GOLDEN, A. M. 1956. Endoparasitism of a spiral nematode on African violet. (Abs.) Phytopathology 46:12-13.

GOLDEN, A. M. and A. L. TAYLOR. 1956. *Rotylenchus christiei*, n. sp., a new spiral nematode species associated with the roots of turf. Proc. Helminth. Soc. Wash. 23:109-112.

GOODEY, J. B. 1952. *Rotylenchus coheni* n. sp. (Nematoda: Tylenchida) parasitic on the roots of *Hippeastrum* sp. Jour. Helminth. 26:91-96.

LORDELLO, L. G. E. 1957. A note on nematode parasites of red Anthurium (*Anthurium andracanum* Lind.), with the description of *Rotylenchus boocki* n. sp. Nematologica 2:273-276.

STEINER, G. 1937. Opuscula miscellanea nematologica, V. Proc. Helminth. Soc. Wash. 4:33-38.

STEINER, G. 1938. Nematodes infesting red spiderlilies. Jour. Agr. Res. 56:1-8.

STEINER, G. and ROWENA R. LEHEW. 1933. *Hoplolaimus bradys* n. sp. (Tylenchidae, Nematoda), the cause of a disease of yam (*Dioscorea* sp.) Zool. Anz. 101:260-264.

On the Occurrence of Gnathostomes in Georgia, U. S. A.

BERT B. BABERO and JACQUELINE R. SHEPPERSON*

Fourteen male and female specimens of adult ganthostomes were recovered from stomach tumors of a raccoon taken in Georgia, U.S.A. Comparative morphological study of these worms with specimens and descriptions of *Gnathostoma spinigerum* Owen, 1836, *G. didelphis* Chandler, 1932, and *G. procyonis* Chandler, 1942, revealed that the worms fitted the descriptions of *G. spinigerum* as given by Baylis (1939) and Baylis and Lane (1920). The present report to the writer's knowledge constitutes the first record of the species from this host.

The raccoon gnathostomes were deeply embedded within small tumors of variable sizes. The worms were surrounded by four gastric ulcers which ranged in diameter from 5–15 mm. (Figs. 1 and 2). A few other erosive areas were observed. Several nodulated lesions were seen on the serosal surface of the stomach. These nodules appeared somewhat similar to those described along the small intestine of opossum by Babero (1957). Histological sections of the stomach showed extensive hemorrhage and hyperemia of many of the mucosal blood vessels. The ulcers extended to depths within the muscularis from which an extensive gastritis ensued, typified by areas of diffused and focal leukocytic and fibroblast proliferation.

A cottonmouth water moccasin (*Agkistrodon piscivorous*), also collected in middle Georgia, was necropsied during routine helminthological investigations. Over one hundred gnathostome cysts were recovered from peritoneum along the abdominal wall. The cysts, which measured less than three millimeters in diameter, were barely perceptible to the unaided eye and could be discerned only by close scrutiny of the tissue. Upon opening a few of the cysts, an actively motile larva was observed in each. The head bulb, cervical sacs, digestive tract, and anal glands were well developed. The average length-of-larva was 3.6 mm. with a maximum average width of 0.29 mm.

*Department of Zoology, Fort Valley State College, Georgia. Supported in part from a faculty research grant.

The writers wish to extend their thanks to Mr. Allen McIntosh for supplying specimens of *Gnathostoma* and to Dr. A. C. Chandler for his comments in relation to this study.

Sexes could not be ascertained with certainty nor could the species be accurately determined. About 20 cysts were orally administered to a young cat and three small white laboratory rats (av. wt., 23 gms.). No gnathostomes were found upon necropsy of these hosts 30 days later. Perhaps the cysts were not in the proper stage of development, since these results are in contrast to those of Chandler (1925), who recovered immature gnathostomes from cats experimentally infected with cysts from *Python reticulatus, Naja hannah* (= *N. bungarus*), and *N. tripudians*. From the investigations of Heydon (1929), Prommas and Daengsvang (1933, 1936, and 1937), Yoshida (1934), and Africa, Refuerzo, and Garcia (1936 a, b), it appears that two intermediate hosts are necessary in the life history of *G. spinigerum*—a microcrustacean (possibly, Cyclops) and a fresh water fish, frog, or snake. According to L. J. Thomas, University of Illinois (personal communication), gnathostome cysts were recovered by him from the musculature of frogs in the Florida region. The finding of encysted gnathostome larvae in a moccasion is further evidence that certain snakes, as well as frogs, of North America also may serve as an intermediate host for parasites of the genus.

LITERATURE CITED

AFRICA, C. M., REFUERZO, P. G. and GARCIA, E. Y. 1936. a. Observations on the life cycle of *Gnathostoma spingerum*. Philipp. J. Sci., 59:513-521.

————. 1936. b. Further observations on the life cycle of *Gnathostoma spinigerum*. Philipp. J. Sci., 61:221-225.

BABERO. B. B. 1957. Some helminths from Illinois opossums. J. Parasitol., 43: 232.

BAYLIS, H. A. 1939. Fauna of British India: Nematoda II. Filaroidea, Dioctophymoidea, and Trichinelloidea. Taylor and Francis, Ltd., London. 274 pp.

————, and LANE, C. 1920. A revision of thee nematode family, Gnathostomidae. Proc. Zool. Soc. London, 20:245-310.

CHANDLER, A. C. 1925. A contribution to the life history of a gnathostome. Parasitol., 17:237-244.

HEYDON. G. M. 1929. Creeping eruption or larval migrans in North Queensland and a note on the Worm *Gnathostoma spinigerum*. Med. J. Aust., 1:583.

PROMMAS, C. and DAENGSVANG, S. 1933. Preliminary report of a study on the life cycle of *Gnathostoma spinigerum*. J. Parasitol., 19:287-292.

————. 1936. Further report of a study on the life cycle of *Gnathostoma spinigerum*. J. Parasitol., 22:180-186.

————. 1937. Feeding experiments on cats with *Gnathostoma spinigerum* larvae obtained from the second intermediate host. J. Parasitol., 23:115-116.

YOSHIDA, S. 1934. Observations on *G. spinigerum* Owen, 1836, cause of esophageal tumor in the Japanese mink, *Lutreola itats*: (Tremminck, 1844) with special reference to its life history. Jap. J. Zool., 1:113-122.

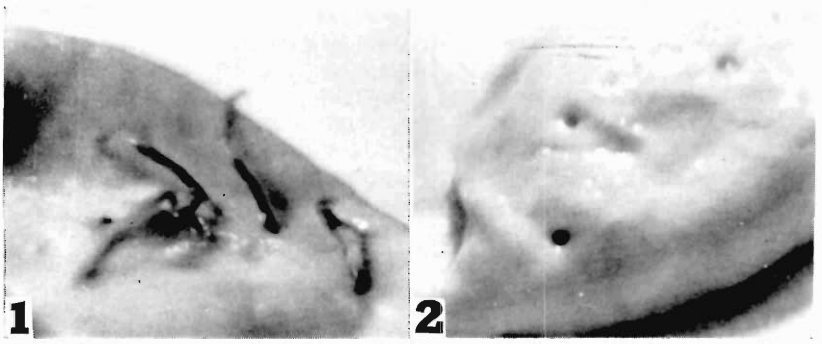

Fig. 1. Shows several adult gnathostomes embedded within the stomach.
Fig. 2. Portion of the stomach showing gastric ulcers.

On *Criconemoides xenoplax* Raski, with Special Reference to its Biology under Laboratory Conditions*

H. A. THOMAS**

Criconemoides xenoplax Raski, 1952 was one of the nematodes frequently encountered in a survey of plant-parasitic nematodes associated with peach roots in New Jersey. Since little appears to be known regarding the biology of the genus and nothing pertaining to this species, laboratory studies as described were conducted during 1956 and 1957.

COLLECTING AND REARING

Before undertaking laboratory studies of *Criconemoides xenoplax* Raski, it became necessary to develop a method for collecting the nematode in sufficient numbers for innoculation and colonization. The following method made possible the recovery of appreciable numbers of the nematodes from large volumes of soil from the field. Enameled metal trays 13 x 21 x 2½ inches were used. Wood frames were constructed which would fit inside the trays; in this case the frame dimensions were 9½ by 18½ inches. A single layer of window screen was tacked across this frame at all edges to form a shallow tray. A layer of gauze is placed on the screen and the soil spread in a ¼ inch layer on the gauze. Then the wood frame is placed in the tray, supported at the corners by small blocks so that the loaded screen does not touch the bottom of the tray. Water is added from the side into the space between the frame and the tray until the soil is submerged.

The writer is indebted to Dr. M. T. Hutchinson, Department of Entomology, Rutgers University for his valuable suggestions during this study.

If the above operations are performed carefully, very little sediment in the sample will result. The tray is left undisturbed for at least twenty-four hours, then the frame is carefully removed and the contents discarded. The nematodes will have worked their way down through the soil and screen and are found in the water beneath. The water in the tray is poured off entirely and allowed to stand for several minutes after which the excess water is decanted; the nematodes having settled to the bottom. Trials indicated that the recovery of nematodes was two to four times greater where a non-ionic wetting agent was added to the water (Atlas Brij 35; 1 ml. per liter of water) before it was poured into the trays than where water alone was used. Sieving and washing of the soil sample are unnecessary. Large and sluggish nematodes more readily work their way through the gauze than through the paper facial tissue often used in funnels. The shallow water apparently provides sufficient aeration so that the nematodes may be recovered alive. Specimens of *C. xenoplax* were removed from the water one by one, examining under a binocular dissecting microscope at 10 X so that populations containing only this species were obtained.

Flats of sandy loam soil were planted with each of three varieties of peach seedlings, Tennessee Natural, Rutgers Red Leaf and Elberta, germinated in the laboratory using the technique of Flemion (1934).

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*From the Department of Entomology, Rutgers University.

Taken from a thesis submitted to the Graduate Faculty of Rutgers University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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Adult specimens of *C. xenoplax* obtained by the above technique were inoculated on peach roots by uncovering a group of feeder roots in different areas of a flat and injecting about 50 cc. of water containing the nematodes onto the roots with a syringe, after which the soil was replaced and wetted. The soil in the flats was held at a temperature of about 20°C. by bench heating coils. Flats were watered daily and commercial fertilizer applied at monthly intervals. Six months after inoculation the numbers of *C. xenoplax* had increased approximately threefold with no significant difference between peach varieties. No other species of *Criconemoides* were found at any time and the identity of specimens as *C. xenoplax* was confirmed by A. L. Taylor, of the Nematology Section, U. S. Department of Agriculture.

VARIATIONS IN THE ADULT STAGE

When the colonies of *C. xenoplax* had become well established, specimens were removed at random for identification and to investigate the range of variation in certain characters of the species, while making certain no other species were present. Adult females were fixed and mounted in Berlese fluid using the method of Reed et al. (1957), and examined for three readily observable characters, viz., spear length, number of annules from vulva to terminus, and total number of annules. According to Raski (1952), the spear of *C. xenoplax* varies from 71—86 microns in length, the vulva is on the 6th—11th annule from the terminus, and the total annules number from 87 to 114. Examination was carried out at 1455X under a phase microscope, measurements being made with the aid of a calibrated micrometer eyepiece.

Most of the data thus obtained fell within the limits set by the author of the species, probably due to the fact that all such specimens examined by the writer were selected from a single colony. Occasional individuals were found to possess spears whose length exceeded 86 microns. However, with regard to the lower limit of spear measurement, 71 microns as defined by Raski (1952), it is this writer's opinion that such a measurement would include among the adults certain specimens in the last larval stage.

INVESTIGATION ON LARVAL STAGES

During the course of the study, specimens were recovered occasionally which were apparently *C. xenoplax* larvae. The examination of the population for the larval stages was therefore, undertaken. To do this, nematodes of all sizes had to be recovered. The method of Caveness and Jensens (1955) was used and since this technique assured virtually complete removal of all nematodes in the soil samples, even the smallest specimens were retained for study. This method, involving centrifugation of the soil sample in sugar syrup solution of adjusted specific gravity (1.25), was sufficiently refined to permit recovery of minute specimens free of silt and debris. One hundred specimens were removed and mounted in Berlese fluid. Measurements were made with a micrometer eyepiece of the overall body length and spear length, and the total number of annules was recorded. The data showed that definite groupings were evident on the basis of size (Table I).

Body length of a specimen can be affected by mounting technique (Taylor, 1936), especially in the case of larval specimens whose body walls are extremely delicate. The possibility of body length variation within a study

TABLE I. Measurements of specimens of a *C. xenoplax* population.

	Spear length (Microns)		Body length (Microns)		Number of Annules	
Larval Groups	Mean	Range	Mean	Range	Mean	Range
I	37	(32-41)	171	(147-209)	104	(93-112)
II	52	(48-59)	329	(235-402)	105	(101-109)
III	68	(63-77)	421	(329-515)	100	(90-108)
Adults	81	(80-92)	676	(529-737)	97	(88-101)

is, however, minimized when all specimens are mounted by the same method and the measurement for body length, together with the spear measurement, provides a useful relationship. The data (Table I) indicate a definite natural discontinuity in the dimensions of various specimens within the population and the intervals thus provided set apart three groups which may be distinct larval stages. But, since the development of individual specimens of *C. xenoplax* could not be followed from egg to adult, these data should not be construed as proof as a certain number of larval stages. The figures do suggest, however, that three molts occur after hatching and this is in substantial agreement with the work of Golden (1956), and Christie and Cobb (1941), who studied the life histories of other nematodes.

On the basis of this study, Raski's (1952) larval description seems to have been based on specimens of the last larval stage preceding the adult stage. The fine longitudinal lines which Raski describes on the posterior edge of the body annules would alone eliminate these as first stage larvae. Even with magnification of 1455X, the writer has never seen these in the first stage larvae or on adults. On the second and third stage these lines, when present, give the posterior edge of the annule a serrated appearance; their absence in some second and third stage larvae is so far unexplained. On the average, the number of annules appear to decrease through successive growth stages to the adult, the latter having the fewest. However, there is considerable variation. Thus the number of annules (96-114) reported by Raski does not identify the stage of his larvae. The length of the spear (59-65 microns), however, seems to most clearly indicate that he was dealing with the third stage.

In most respects, the larvae generally resemble the adults in basic morphology except that neither the ovaries nor the vulva could be located in the larvae and the larvae were considerably more hyaline than the adults. Taylor (1936) suggests that the female of the genus *Criconemoides* may be syngonic, with the ovaries forming first the sperms and then the ova, while Raski (1952) states that males of *C. xenoplax* are rare. No males specimens of *Criconemoides xenoplax* were ever found during the course of the present study.

BIOLOGICAL STUDIES

For detailed studies of the nematode, newly germinated peach seedlings were maintained in petri dishes. The seedlings were planted in a finely divided sedge-peat medium (trade name, Hyper-Humus), where the roots of the seedlings usually grew down to the glass bottom of the dish and then spread along the glass-soil interface. Nematodes were added in such a way that they would be found along these visible roots. The habits of the nematodes were then observed by inverting the dishes under a binocular dissecting microscope at 90X. When not under observation, the seedlings were maintained at 20°C. in an illuminated constant-temperature cabinet.

EGG STAGE

The female deposits eggs singly every 2 to 4 days. The eggs are never found in a clutch, but no egg was ever found or seen to be deposited any distance from the roots. Due to the tendency of the adult female to lie with the posterior end of the body recurved toward the root when feeding and the apparent habit of depositing eggs only when near the host, the eggs are ordinarily found quite close to the roots. Eggs were found on the surface of the roots or in the surrounding medium, but the egg evidently lacks any adhesive and no eggs were seen attached or inserted. Shortly before the egg is laid, it may be seen in the lower end of the ovary. On occasion, from ten to twenty immature eggs may be seen in the anterior portion of the ovary. A full-sized freshly deposited egg measures about 33 by 66 microns, has a milky white appearance, and is quite smooth over its entire surface. These measurements agree with those of Taylor (1936), who states that the egg is about as long as the width of the adult's body, and the width of the egg is about half its length.

No nematode was ever observed to deposit eggs until it had been adjacent to the roots of the host and taking food for several days. This fact would suggest that feeding or some associated function is necessary to stimulate egg development and deposition.

The chorion of the egg is an hyaline membrane. In certain stages, changes in configuration and cell division may be observed within the egg. Toward the end of embryonic development the larvae may be seen folded double within the confining membrane. From the time of egg deposition to hatching, development took approximately fifteen days under the conditions of this study. Upon hatching, the larva is found to measure slightly over 130 microns in length which would roughly correspond to its being folded double in an egg slightly over 65 microns in length.

OBSERVATIONS ON FEEDING ACTIVITY

According to Taylor (1936), nematodes of the genus *Criconemoides* make their way about by alternately extending and compressing their body along the longitudinal axis; the retrose segmental edges of the annules providing traction. These observations were confirmed in this study. Observations made after inoculation of specimens of *C. xenoplax* around peach roots showed that this nematode is slow to reach the immediate root zone. Occasional specimens of C. *xenoplax* required as much as several days before they began to feed, and many specimens never reached the roots of the host at all unless the nematodes were initially inoculated quite close to the roots. When a specimen of *C. xenoplax* reached the area immediately adjacent to the peach root, it was seen to begin seeking physical contact with the host by methodical trial-and-error probing with the head. By this probing, the nematode evidently locates the root epidermis and, possibly, zones favorable for penetration. That this probing must precede the act of feeding is apparent when it is noted that no stylet motion takes place until the exploratory probing is completed. It may be reasonable to assume that when the lip region makes contact with suitable host material, the sensory receptors of the lip region are stimulated to initiate appropriate feeding movements. Once contact with the surface of the host has been made by the lip region, the stylet is seen to begin probing the surface of the host. During exploratory probing, the stylet is thrust short distances, slowly

and deliberately, while the head is moved from side to side and up and down; the lip region being held fairly close to the region under attack. Specimens of *C. xenoplax* were seen to feed and/or congregate at root tips as well as along roots, with no apparent partiality toward any region, as contrasted with *Pratylenchus* and *Meloidogyne* (Linford, 1939).

During a brief interval of 1 to 2 minutes, the nematode begins final insertion of the stylet, and all other body movements cease. At this time the nematode is usually settled in a slightly curved position along an axis more or less parallel to the root. Insertion of the stylet into the host is reported to be accomplished with the aid of the muscles attached to the spear at its basal end. The process of inserting the stylet is slow and deliberate, often requiring several minutes, but once insertion is begun the stylet is not withdrawn to any observable extent. That the nematode requires much traction for its body in order to insert the stylet is doubtful. In one instance, the root was penetrated while the nematode was lying on moist glass, where no other support was available. During the probing activity the stylet base was frequently seen to move out of the mid-longitudinal axis of the body, which indicates that the stylet tip can leave the body at an angle. On a number of specimens the stylet was seen to leave the lip region at an angle. Very often the stylet is thrust out of the body until the junction between the basal portion and the anterior portion of the stylet is on a line with the lip region. Once in this position, the basal third of the stylet apparently remains lying along the longitudinal axis of the head, while the anterior part may be penetrating the host at an angle. The stylet is thus flexible at this point. The stylet of an adult *C. xenoplax* is very often seen extruded about two-thirds of its length, or about 57 microns.

During feeding, observation can be made through the body wall of the nematode and in certain instances, the crescentic valve can be seen pulsating regularly. Individual *C. xenoplax* specimens have been observed to feed continuously at one point for a period of 18 hours, and under natural conditions feeding probably continues uninterrupted for even longer periods of time. All post-embryonic life stages were observed feeding on peach roots of the Tennessee natural variety. With the exception of feeding and some moulting, observations on the larvae were limited. Female adults of *C. xenoplax* were observed to remain alive for more than 2 months under laboratory conditions.

LITERATURE CITED

CAVENESS, F. E. and J. JENSENS. 1955. Modification of the centrifugal flotation technique for the isolation and concentration of nematodes and their eggs from soil and plant tissue. Proc. Helm. Soc. Wash. 22: 87-89.

CHRISTIE, J. R. and G. S. COBB. 1941. Notes on the life history of the root knot nematode, *Heteroderma marioni*. Proc. Helm. Soc. Wash. 18: 106-108.

FLEMION, FLORENCE. 1934. Dwarf seedlings from non-after ripened embryos of peach, apple and hawthorn. Contrib. Boyce Thompson Institute. 6: 205-209.

GOLDEN, A. M. 1956. Taxonomy of the spiral nematodes and the developmental stages and host-parasite relationships of *Rotylenchus buxophilus* attacking boxwood. Univ. of Md. Bull. A-85.

LINFORD, M. B. 1939. Attractiveness of roots and excised shoot tissues to certain nematodes. Proc. Helm. Soc. Wash. 6: 11-18.

RASKI, D. J. 1952. On the morphology of *Criconemoides* Taylor, 1936 with descriptions of six new species. Proc. Helm Soc. Wash. 19: 85-99.

REED, J. P., M. T. HUTCHINSON, and S. R. RACE. 1957. A new permanent method for mounting plant parasitic nematodes. U.S.D.A. Pl. Dis. Reptr 41: 25-26.

TAYLOR, A. L. 1936. The genera and species of the *Criconematinae*. Am. Micro. Soc. 55: 391-421.

The Influence of Certain Mineral Elements on Emergence of Golden Nematode Larvae*

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It is generally recognized that many members of the Solanaceae family secrete from their roots a substance which stimulates the emergence of golden nematode (*Heterodera rostochiensis* Wollenweber) larvae. Studies have been reported relating to the concentration of the natural hatching stimulant and its possible chemical nature (Marion, et al., 1949; Massey and Neal, 1951, 1953; Janzen and van der Tuin, 1956; Widdowson and Wiltshire, 1958) and attempts have been made to synthesize active compounds (Marion, et al., 1949; Russell et. al., 1949). Hatching response to root diffusates was studied by Fenwick, 1950, 1952.

During our attempts to concentrate the natural hatching stimulant, larval emergence was observed to decrease after inorganic matter was removed from tomato (*Lycopersicon esculentum*) root diffusates by the use of ion exchange resins. However, combining the cations (as chlorides) eluted from the cation resin with the effluent resulted in a hatching response equivalent to the initial diffusates. Therefore, a study was undertaken to determine the influence of mineral elements on larval emergence.

MATERIALS AND METHODS

The method of obtaining leachings and assaying for hatching activity were the same as described in a previous publication (Robinson and Neal, 1956). The soil in which tomato plants were growing was subjected to a mist of tap water for about 8 hours, and the liquid that drained through the soil was collected as a measure of normal leachings. For assay, usually 100-150 cysts per petri dish were incubated at 20°C with 25 ml. of the solution to be tested. Distilled water was used to make the respective solutions. Hatched larvae were counted after the incubation period, and the results are expressed as larval emergence per cyst.

Leachings were partially "deionized" by first passing them over Amberlite IR-120 cation exchange resin in the hydrogen state. If the effluent were not at approximately pH 4, it was adjusted to this value with HCl and then passed over a column of Amberlite IR-4B anion exchange resin in the hydroxide state. These deionization steps were repeated until the effluent had a specific conductance of not greater than 20 micromhos (reciprocal ohms) when adjusted to 50 micrograms of solids per ml. The solution was then lyophilized and labeled "deionized" leachings. This preparation would be expected to contain neutral as well as weakly acidic compounds since Amberlite IR-4B is a weak basic resin and the acidity of the influent was adjusted to suppress ionization of weak acids.

The cations and anions were eluted from the IR-120 and IR-4B columns with 1 N HCl and 1 N NH_4OH, respectively, after which the eluates were lyophilized. Thus, the cations in the original leachings were obtained as their

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chlorides and the anions as their ammonium salts. Approximate dry weights expressed as mg. per liter of the various fractions were as follows: original leachings, 800; "deionized" leachings, 20; cation chlorides, 1160; ammonium anionates, 200.

The influence on larval emergence of heavy metal ions present in normal leachings was studied conveniently by extracting these cations from leachings with a 0.01% solution of dithizone in chloroform. After separating the two layers, the aqueous solution was extracted with chloroform to remove any residual dithizone; the water phase was separated and then aerated to remove the last traces of chloroform. The cations were recovered from the chloroform solution containing the metal chelates by extracting them with 1 N HCl after which the aqueous solution was lyophilized. The influence of zinc and cadmium ions on larval emergence was also determined.

RESULTS

Hatching activity as measured by larval emergence per cyst (using cysts from England) of four different batches of leachings after "deionizing" ranged from 41—83% of the activity of the respective original leachings. The cations eluted from the Amberlite IR-120 column possessed negligible stimulatory activity; however, when they were added back to the respective "deionized" leachings, larval emergence was equivalent to the original leachings. Larval emergence per cyst in the above assays was quite low (max. 9 larvae per cyst); but, the fact that significant differences (5% level) were obtained when the cation chlorides were added to the "deionized" leachings indicated that inorganic ions play an important role in larval emergence. Therefore, attempts were made to obtain a known mineral solution which might be substituted for the variable kinds and amounts of cations occurring in leachings. A mixture of salts (Salts A, Table 1) containing calcium, magnesium, sodium and potassium chlorides when added to "deionized" leachings resulted in an increase in larval emergence which was equivalent to or greater than that produced by adding back the cation chlorides which had been eluted from the cation exchange resin. Experiments using cysts from Belgium (1955 and Sand Dune, 1956) which gave a greater larval emergence than did the cysts from England confirmed this stimulatory influence of Salts A on larval emergence (Fig. 1). The respective pairs of curves (Fig. 1) show the increase obtained in larval emergence in the presence of Salts A and varying levels of "deionized" leachings at pH 6.5–7.0. Three different batches of leachings were used. Data for pairs of curves I and IA were obtained from the same assay and indicate that the differences obtained after a one-week incubation period remained significant after the longer period of incubation. The means of the respective pairs of curves IA and II, and I and III are significantly different at the 1% and 5% level, respectively.

In view of the findings of Robinson and Neal (1956) that the natural hatching factor possesses greater activity at pH 3 than at neutrality, the

Table I. Composition of Salts A

	μg. per ml.*
Potassium chloride	9.3
Sodium chloride	12.4
Magnesium chloride	27.4
Calcium chloride	450.8

*Concentration in assay medium.

influence of Salts A in a medium at pH 3 was determined. Data (Table 2) show the very highly significant increase (L.S.D., 0.5 = 56.2) in larval emergence at this lower pH value as a result of adding Salts A to "deionized" leachings. The concentration of leachings in this experiment (400 microg. per ml.) was equivalent to a 16- to 20-fold concentration of normal leachings.

Individual components of Salts A and various combinations of them failed to produce an emergence of larvae equivalent to the complete mixture; however, potassium appeared to stimulate hatching more than any other single component of the salt mixture. Hoagland's plant nutrient solution was no more effective in stimulating larval emergence than Salts A.

Evidence concerning the inhibitory influence on larval emergence of heavy metallic ions present in some leachings was indicated by the observations that dithizone extracted leachings possessed greater hatching activity (using cysts from England) than normal leachings, and, when the cations removed by dithizone were added to the dithizone extracted solutions, hatching activity was lowered to approximately the initial value for the respective original leachings. For example, when normal leachings and cysts from England were used, larval emergence was 25 larvae per cyst (pH 6-7, incubation—one week), whereas after dithizone extraction this value increased to 38 (signifi-

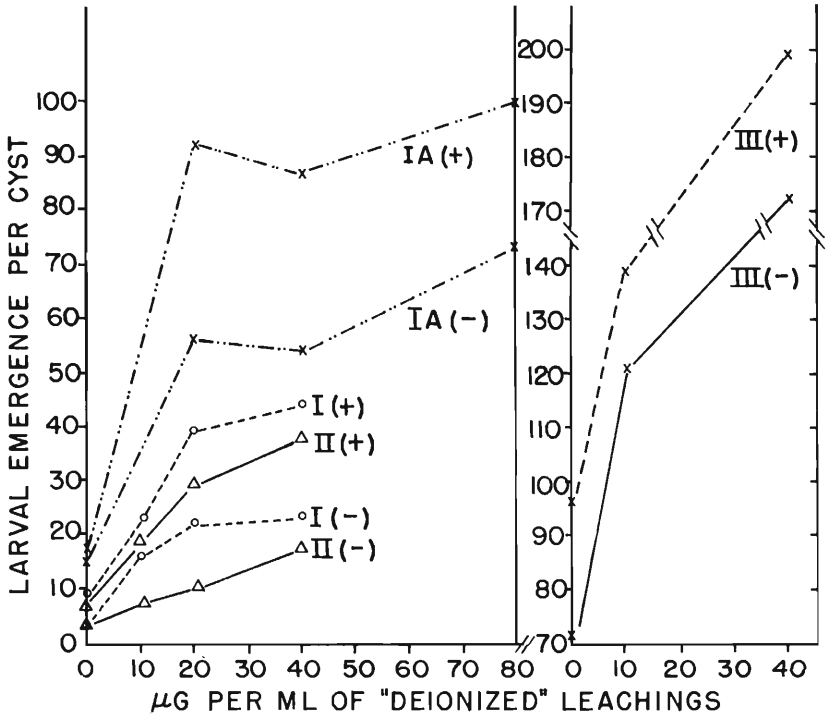

Figure 1. Influence of Salts A on larval emergence using "deionized" leachings at pH 6—7 and Belgium (1955) cysts. The (+) or (—) indicate that Salts A were or were not added to the assay medium. The average of duplicate assays were used to plot curves I, IA and II; whereas, the average of four replicate assays were used to plot curves III. Incubation period for curves I and II was 7 days, and for curves IA and III it was 14 days.

cant 5% level). When the extracted leachings plus the extracted metal ions were assayed, larval emergence was 26 larvae per cyst. Zinc and cadmium were found to exert a pronounced inhibitory influence on larval emergence. The addition of zinc, as sulfate, at a level of 2 microg. per ml. to dithizone extracted leachings resulted in a decrease in larval emergence of about 50%. These data are in accordance with the observations of Ellenby, 1942, concerning the beneficial effect of zinc when it was added to nematode infested soil in which potato plants were growing. The element cadmium, as chloride, was found to decrease larval emergence in normal leachings about 57% and 95% at concentrations of 0.25 and 0.1 microg. per ml., respectively.

DISCUSSION

The observed stimulatory or inhibitory effect of cations upon larval emergence is of particular interest as related to the biological assay of golden nematode hatching stimulants. From the data presented, it is apparent that misleading information relative to the hatching activity of solutions may be obtained unless the medium is defined with respect to cations and hydrogen ion concentration. The use of tap water as the assay medium introduces unknown and varying concentrations of metallic ions which may influence larval emergence differently from one time to another or at different locations. It has been observed by Nebel, 1926, that stimulatory compounds were more effective in soil solution than they were in pure water; however, in view of the results presented above, this would be true only insofar as the concentration of heavy metallic cations were not high enough to cause inhibition of larval emergence. The inhibitory influence on larval emergence using the first collections of leachings has been reported by Johnson and Townsend, 1949, and confirmed in our laboratory, Massey and Neal, 1951. The former investigators suggested that ammonia was the cause of this inhibition. From the data obtained with dithizone extracted leachings, it is possible that high levels of heavy metallic ions may also have contributed toward this inhibition.

SUMMARY

Larval emergence from golden nematode cysts is influenced by the kind and concentration of cations present in the solution. A mixture of sodium, potassium, magnesium and calcium chlorides when added to tomato root leachings from which the cations had been removed resulted in a stimulation of larval emergence equal to or greater than that obtained by the addition of all of the cations removed from the original leachings. Larval emergence may be inhibited by the high concentration of heavy metal cations normally present in some leachings and by relatively low amounts of zinc and cadmium.

It is suggested that the assay medium for testing golden nematode hatching stimulants be adjusted to pH 3 with HCl and contain Salts A in addition

Table 2. Influence of Salts A on Larval Emergence in Solution at pH 3.

Treatment*	Larval Emergence per cyst**
1. Water plus Salts A	27
2. "Deionized" leachings (400 microg./ml.)	201
3. "Deionized" leachings (400 microg./ml.) plus Salts A	300

* All solutions were acidified to pH 3 with HCl.

** Average of four replicates (50 cysts each) using Sand Dune, 1956, cysts from Belgium. Incubation period, 14 days. L.S.D. 0.5%=56.2.

to leachings, from which the cations have been removed, or the compound to be tested.

LITERATURE CITED

ELLENBY, C. 1942. Trace-elements and "Potato-Sickness." Nature, London, 149:50.

FENWICK, D. W. 1950. Investigations on the emergence of larvae from cysts of the potato-root eelworm *Heterodera rostochiensis*. 2. The form of the hatching curve. J. Helminth, 24:75-86.

———— 1952. The bio-assay of potato-root diffusate. Ann. Appl. Biol., 39: 457-467.

JANZEN, G. J. and F. VAN DET TUIN. 1956. The unknown hatching agent for the potato root eelworm. Nematologica, 2: 126-137.

JOHNSON, L. R. and W. N. TOWNSEND. 1949. The inhibition of hatching of potato root eelworm (*Heterodera rostochiensis* Wollenweber) in patrially sterilized soil. Ann. Appl. Biol., 36:504-512.

MARION, D. H., P. B. RUSSELL and A. R. TODD. 1949. The potato eelworm hatching factor. 6. Attempts to prepare artificial hatching agents. Part II. Some active arylidene—$\Delta\beta$:8 butenolides and related compounds. Biochem. J., 45:533-537.

MARION, D. H., P. B. RUSSELL, A. R. TODD and W. S. WARING. 1940. The potato eelworm hatching factor. 3. Concentration of the factor by chromatography. Observations on the nature of eclepic acid. Biochem. J., 45:524-528.

MASSEY, JR., L. M. and A. L. NEAL. 1951. Investigations on the golden nematode hatching factor. Fed. Proc., 10:222.

———— 1953. Investigations concerning the hatching factor of the golden nematode of potatoes (*Heterodera rostochiensis* Wollenweber). J. Wash Acad. Science, 43:396.

NEBEL, B. 1926. Ein Beitrag zur Physiologie des Rübennematoden *Heterodera Schachtii* von Standpunkt der Bekämpfung. Kühn Archiv., 12: 38.

ROBINSON, T. and A. L. NEAL. 1956. The influence of hydrogen ion concentration on the emergence of golden nematode larvae. Phytopath. 46:665.

RUSSELL, P. B., A. R. TODD and W. S. WARING. 1949. The potato eelworm hatching factor. 5. Attempts to prepare artificial hatching agents. Part I. Some furan derivatives. Biochem. J., 45:530-532.

WIDDOWSON, E. and G. H. WILTSHIRE. 1958. The potato eelworm hatching factor. Ann. Appl. Biol., 46:95-101.

Trophurus minnesotensis (Caveness, 1958), n. comb.

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Upon the publication of my description of a new genus and new species in 1958, the paper by Loof (1956) was called to my attention. It is agreed that *Clavaurotylenchus* Caveness, 1958 is a synonym of *Trophurus* Loof, 1956. However, the re-examination of both freshly collected and preserved specimens of *T.* (C.) *minnesotensis* and comparison with the paratype of *T. sculptus,* kindly loaned by Dr. M. W. Allen, reveals specific differences between these two. Thus, the former becomes *Trophurus minnesotensis* (Caveness, 1958), n. comb. The basal esophageal bulb is elongate-ovate and a hemizonid is evident, in the fresh specimens, 3 to 5 annules posterior to the excretory pore of *T. minnesotensis* whereas the basal esophageal bulb is subpanduriform and no hemizonid has been found in *T. sculptus.*

LITERATURE CITED

CAVENESS, FIELDS E. 1958. *Clavaurotylenchus minnesotensis,* n. gen., n. sp. (Tylenchinae: Nematoda) from Minnesota. Proc. Helm. Soc. Wash. 25:122-124.

LOOF, P. A. A. 1956. Trophurus, A New Tylenchid Genus (Nematoda). Versl. en Meded. Plantenz. k. Dienst 129 (Jaerboek 1955:191-195).

A Technique for Oral Infection of Earthworms

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Since earthworms are obligate or facultative intermediate hosts for several species of nematodes, it is frequently necessary to infect them for use in experimental work. The usual method is to establish a culture of annelids and add to it large numbers of eggs or larvae. However, when only a few eggs or larvae are available, or when each earthworm is to receive a known number, or when it is desirable to know the exact time of infection, this "mass infection" technique is not satisfactory. Dunn (1955) used capillary pipettes to infect earthworms with small numbers of *Metastrongylus* eggs. A method which has been successfully employed by the writer for the infection of earthworms with larvae of *Stephanurus dentatus* and eggs of *M. apri* and *Choerostrongylus pudendotectus* is described herein.

The infective material is drawn up into a tuberculin syringe fitted with a 20-gauge needle which has been blunted and smoothed at the tip. The earthworm is prepared for feeding by first washing with tap water and then briefly immersing in 20% ethyl alcohol. On being placed in alcohol, *Eisenia foetida* immediately discharges a cloud of yellow pigmented material and exhibits a series of writhing and coiling movements followed by relaxation in about 30 to 60 seconds. The specimen is then removed, washed quickly in tap water, and placed on a paper towel. The tip of the needle is inserted into the mouth and about a quarter of an inch down the esophagus. The anterior end of the earthworm is grasped lightly with fine forceps, as shown in Figure 1, and the infective material introduced by gentle pressure on the plunger. Mature *E. foetida* can usually take 0.05 to 0.1 ml., if this volume is administered slowly. If the procedure is successful, an increased turgidity of the anterior portion of the worm will be noted. The annelid is then placed

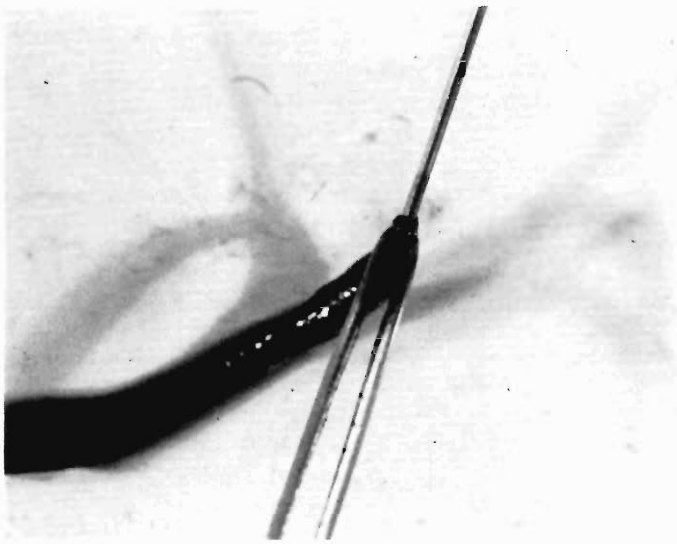

Figure 1. *Eisenia foetida* in position for oral infection. (X 1.5)

on moist filter paper in an individual petri dish or small wide-mouthed bottle. Deaths due to overexposure to alcohol or injury during feeding and handling usually occur within 24 hours. To date, the survival rate has been 87% of 310 *E. foetida* infected by the use of this technique.

LITERATURE CITED

DUNN, D. R. 1955. The culture of earthworms and their infection with *Metastrongylus* species. British Vet. Jour. 111: 97-101.

A Study of the Intestinal Helminths of the Southern Crow *(Corvus brachyrhynchos paulus)* in Virginia.

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Recent concern with the geographical and ecological distribution of populations of the southern crow in relation to their food habits has stimulated considerable interest in this bird. As the crow is omnivorous, its diet includes a number of invertebrate and vertebrate animals which may serve as intermediate or reservoir hosts for its intestinal helminths. During 1956-57, the intestines of 103 crows were examined to ascertain which helminths frequent the lower part of the alimentary tract of the southern crow in Virginia. It was found to harbor representatives of the Cestoda, Nematoda, Trematoda, and Acanthocephala.

COLLECTION OF MATERIAL

Crows were collected by shooting in the fall, winter, and spring. Dissections were made within a few hours after death. The intestine and cloaca were removed and examined with the aid of a stereoscopic microscope. The tissue was frequently macerated and, before fixation, the living worms were relaxed in physiological saline solution or several changes of fresh water. The specimens were fixed in F.A.A. (formalinacetic acid-alcohol), 10 per cent formalin, or Carnoy's fixative; stained in Harris' or Ehrlich's haematoxylin, and mounted in balsam. Sectioned material was stained with Delafield's haematoxylin.

RESULTS

A total of 62 of the 103 crows examined were infected with seven genera and eight species of helminths. Cestodes infected 54% of the hosts with *Anomotaenia constrict* (Molin, 1858) 35 infections, *Hymenolepsis variabile* (Mayhew, 1925) 25 infections, *Paruterina reynoldsi* (Daly, 1958) 4 infections, *Cladotaenia* sp. 1 infection; nematodes infected 9% with *Porrocaecum ensicaudatum* (Zeder, 1800) 8 infections, *Porrocaecum* sp. 1 infection; trematodes infected 6% with *Echinostoma revolutum* (Frölich, 1802) 6 infections; acanthocephalans, *Mediorhynchus grandis* (Van Cleave, 1916) 1 infection. Prevalence of infection with *Anomotaenia constricta* and *Hymenolepsis variabile* was comparatively high and their monthly incidence percentages were remarkably constant.

REFERENCES

DALY, E. F. 1958. A new dilepidid cestode, *Paruterina reynoldsi*, from the southern crow, *Corvus brachyrhynchos paulus* Howell. Proc. Helm. Soc. Wash. 25: 34-36.

WARD, J. W. 1935. Some parasites of crows of central Oklahoma (Preliminary report). Okla. Acad. Sci. No. 15.

The Life History of *Hemicycliophora arenaria* Raski (Nematoda: Criconematidae)*

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Hemicycliophora arenaria Raski, 1958, an ectoparasitic nematode, was first found attacking rough lemon (*Citrus limonia* Osbeck) in Southern California by Van Gundy (1957). The disease symptom was enlargement of the terminal and lateral root tips which resembled small knobs. Later Van Gundy (1958) showed that the enlargement of the root tips was due to an increase in cell-division. The cells in the area of feeding also showed hypertrophy of the nuclei suggesting the secretion of one or more enzymes by the nematode. Rough lemon seedlings which had been inoculated with 250 nematodes and grown for 5 months at 30°C were 35 per cent smaller than the non-inoculated trees. The nematode population increased at the rate of 1 to 640 during this period.

Rough lemon is the only reported host of this species, however, host range studies that are underway and to be reported later show that *H. arenaria* also parasitizes and reproduces on a number of herbaceous plants. Reproduction was extremely high on tomato roots. This is mentioned at this time because tomato seedlings were used in parts of this study. This also illustrates the potentiality of this species as a plant parasite. Since there was no previous information concerning the life history of the genus *Hemicycliophora* de Man, it seemed important to study this phase of *H. arenaria*.

MATERIALS AND METHODS

The development within the egg was followed by allowing gravid females to lay eggs in depression slides filled with fresh tap water. The adult nematodes were removed and the eggs incubated in moisture chambers at 28-30° C. The water covering the eggs was changed with a micro-pipette every day. The eggs were examined under a stereoscopic microscope (112X) at 6-8 hour intervals and the hatched larvae were removed to other depression slides filled with tap water and observed every 24 hours. The water on the larvae was changed every 3 days.

The life cycle was completed only when the nematodes were allowed to feed upon roots of a suitable host. For these studies tomato seedlings were easier to grow and manipulate than citrus seedlings. Water suspensions containing 100 one-day old larvae were added to half-pint paper cups each containing a tomato seedling in soil. They were maintained at 30° C in a temperature tank. The seedlings and soil were checked at 3-day intervals for the various stages of the nematode. Tomato seedlings similar to those described above were inoculated with 30 hand-picked fourth-stage female larvae to determine if reproduction could take place in the absence of males. Additional observations also were made in Petri dishes containing water agar and sterile tomato seedlings. Eggs that had been deposited on water agar by females previously rinsed three times in sterile water were then transferred to the dishes containing the tomato seedlings. These eggs hatched and developed eventually into young females and males. Unfortunately, the cultures were lost before they completed the entire life cycle.

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Various stages in the life history were studied from mass collections of hand-picked greenhouse colonies maintained on rough lemon and tomato seedlings. Both fresh and fixed specimens were examined. Nematode measurements were made on specimens fixed in 5 per cent formalin and mounted in aniline blue-lactophenol. This stain facilitated the differentiation of reproductive tissue in the larval stages. Twenty specimens of each stage were measured.

RESULTS

THE EGG AND ITS DEVELOPMENT: Gravid females usually deposited from one to many eggs within 6 hours in depression slides filled with tap water. The number of eggs laid and per cent that hatched are given in Table I. Newly laid eggs were one- to two-celled, and averaged 58 x 156 microns in size. Egg membranes were thin-walled and flexible, especially when larvae were moving about within them. The eggs were deposited singly by the females. Each egg was covered with a sticky substance by which it adhered tightly to the glass slide. After six hours the eggs were four-celled and proceeded rapidly through successive divisions, hatching in 3-5 days. The first-stage larvae within the egg did not have a stylet. The first molt occurred in the egg about 12 hours prior to hatching. Shortly after the first molt, a stylet was observed (Fig. 1, A).

Several second-stage larvae were observed hatching from eggs, each ensheathed in its first larval cuticle.

SECOND-STAGE LARVAE: L = 281-333 microns; W = 15-19 microns; a = 17.6 (16.0-19.3), b = 2.8 (2.6-2.9), c = 7.9 (6.9-8.9); Stylet = 49-60 microns; Esophagus = 99–115 microns; Tail = 34–48 microns. The excretory pore was located anterior to the basal bulb (Fig. 1, A). The conspicuous hemizonid was located 2 annules above the excretory pore. The reproductive system consisted of 4 cells. The tail shape is illustrated in Fig. 1, A.

The second-stage larvae required a feeding period of about two days before the second molt took place. Second-stage larvae held in fresh water in depression slides became exhausted and died within 1 week.

The second-stage larvae moved directly from the eggs to the roots of small tomato seedlings on agar plates. They migrated along the root until a root tip was reached where they began feeding. Larvae were observed feeding only in the region near the root cap with their stylets inserted several cells deep. They often withdrew their stylets, moved about, and then resumed feeding at another location. No killing of the cells was observed, but root elongation terminated after one day of feeding by the larvae. The valvular

TABLE 1. Number of eggs laid by young gravid females of *Hemicycliophora arenaria* Raski and the number of eggs hatching at 28°–30°C.

	No. of Females	Total No. of Eggs 6 hrs. old	Av. / Female	% hatch			Not hatching after 7 days
				3 days	4 days	5 days	
Test 1	56	324	5.7	17	74	6	3
Test 2	68	429	6.3	25	59	10	6
Test 3	28	212	7.5	26	56	12	6
Test 4	39	220	5.6	12	69	12	7
Totals	191	1185	6.2	20	65	10	5

apparatus of the esophageal bulb was in continuous motion during feeding periods up to 15 minutes. No rest periods were observed.

The nematodes ceased feeding while molting and moved away from the root tip into the agar. They became inactive, dissolution of the posterior portion of the stylet occurred and the nematodes turned dark throughout, shrinking away from the old cuticle. The process of molting took approximately 24 hours. The molt to the third-stage larvae was completed before the cuticle of the first molt was lost.

THIRD-STAGE LARVAE: L = 428–532 microns; W = 21–25 microns; a = 20.7 (17.4-23.7), b = 3.6 (3.1-3.9), c = 10.3 (9.2-11.4); Stylet = 68-81 microns; Esophagus = 116-147 microns; Tail = 42-56 microns. The third-stage larvae were distinctly larger in size and the spear and esophagus more advanced than the second-stage larvae. The excretory pore was located opposite the basal bulb. There was no change in number of the genital primordia. The tail shape of the third-stage larvae differed from that of the second-stage larvae (Fig. 1, B).

This stage, like the second, failed to develop any further if removed from the host. The nematodes remained active for 2-3 weeks in fresh water. A feeding period of 2 days usually initiated the third molt. The cuticle of the second molt was not lost until after the completion of the third molt. Shortly after completion of the molt the male and female became differentiated.

FOURTH-STAGE MALE LARVAE: L = 526–601 microns; W = 27–30 microns; a = 19.1 (18.1–21.2), b = 4.3 (4.1–4.8), c = 11.3 (10.6–13.4); Stylet = 67–80 microns; Esophagus = 112-132 microns; Tail = 45-52 microns. In size and shape the fourth-stage male larvae were intermediate between the third-stage larvae and the fourth-stage female larvae. They were differentiated by the collapsed esophagus, weak basal knobs of the stylet, and the development of the reproductive tissues. Under the stereoscopic microscope the fourth-stage male larvae appeared dark throughout. The genital cells at the anus enlarged during molting and later developed into the spicule (Fig. 1, C). Division of the spermatocytes occurred at the same time. The tail shape was similar to that of the third-stage larvae. During the fourth molt the remaining fragments of the stylet disappeared and a new stylet did not develop in the adult male. No feeding was observed after the third molt, and development into adult males was complete in 4-6 days.

FOURTH-STAGE FEMALE LARVAE: L = 609–726 microns; W = 27–37 microns; a = 23.5 (20.1–26.3), b = 4.1 (3.9–4.3), c = 13.4 (11.8–51.0); Stylet = 76–92 microns; Esophagus = 135-177 microns; Tail = 45-55 microns. These larvae were much longer and more fully developed than the male larvae. There was little change in the excretory pore or in the hemizonid from the third-stage larvae. The genital cells located 10-12 annules anterior to the anus began enlarging and developing into a vulva and uterus (Fig. 1, E). The uterus connected the oocytes which had undergone 3-4 divisions, with the area around the vulva. The tail shape is the same as that of the third-stage larvae.

This stage also had to feed upon a suitable host before further development. These nematodes remained alive for 2-3 weeks in fresh water without molting. A two-day feeding period initiated the fourth molt.

ADULT FEMALES: L = 825–975 microns; W = 42–48 microns; a = 22.3 (19.5-26.5), b = 5.1 (4.7-5.4), c = 18.5 (14.5-20.8); Stylet = 95-105 microns; Esophagus = 173-200 microns; Tail = 45-55 microns. These measurements are of young gravid females. A hemazonid was observed in young females 2-3 annules anterior to the excretory pore.

A significant characteristic of the adult female was the production of a sixth cuticle. This extra cuticle developed shortly after the fourth molt and prior to emergence from the fourth larval cuticle (Fig. 1, F and G). Both adult cuticles were attached at the head and vulva, each had a vulva opening and remained on the females throughout their adult life. The stylet was not dissolved and lost during the development of the sixth cuticle as in the other larval stages.

If feeding was resumed immediately after the fourth molt, 4-8 mature eggs were laid in 24-48 hours. The elapsed time from egg to egg in the course of these studies was approximately 15-18 days at 30° C. Adults remained alive and active in fresh water for 4-5 weeks without feeding.

ADULT MALES: L = 674-738 microns; W = 20-23 microns; a = 32.8 (30.7–36.0), b= 5.3 (5.0–5.6), c = 8.1 (7.4–9.0); Esophagus = 128–140 microns, Tail = 75–85 microns. There was no sixth cuticle. The tail shape is entirely different from that of any other stage (Fig. 1, D). In these studies the adult males did not live for more than 2 weeks in fresh water.

Males were not essential for reproduction. Each of five tomato seedlings were inoculated with 30 hand-picked fourth-stage female larvae. Approximately one-fourth of these larvae developed into adult females. Ten days after inoculation second- and third-stage larvae were recovered, indicating that the unfertilized females produced viable eggs.

DISCUSSION

The life history of *H. arenaria* is particularly interesting in view of the recent emphasis on the pathogenicity of ectoparasitic nematodes. The disease cycle of this large group of ectoparasites, although simpler, is probably influenced more by the soil environment than are the endoparasites and the sedentary ectoparasites. The life stages follow the expected development with some unique variations. As the eggs of *H. arenaria* matured within the female they were deposited singly about the root tip, possible over an area of 3 to 4 cm. Each egg was covered with a sticky substance which may protect the eggs from partial dessication and removal from the root zone by water. The egg stage usually lasted 3-5 days. After hatching the existence of the second-stage larvae was dependent upon locating a suitable host root-tip because without food they soon perished. Growth and development of each larval stage required a period of feeding upon a suitable host. As additional protection from its environment the larvae and adult females are surrounded by two cuticles during their life stages.

The development of the larval stages was similar to that of other plant parasitic nematodes. There were four larval molts, the first of which was in the egg. As the larval stages molted, the base of the stylet was dissolved and the anterior portion cast off with the larval cuticle. A new stylet did not develop in the adult male. Paetzold (1958) also found that the stylet of the male of *Hemicycliophora typica* (de Man) was cast off with the last larval cuticle and did not develop in the adult male.

The ratios of de Man helped separate the different stages. Overlap in measurements could not be avoided since it was impossible to get all the larvae of any one stage at the same age. A period of several hours made considerable difference in the development of this nematode. Each stage was easily recognized by using the ratios, overall length, development of esophagus (degenerate in male larvae), shape of tail and development of reproductive tissues.

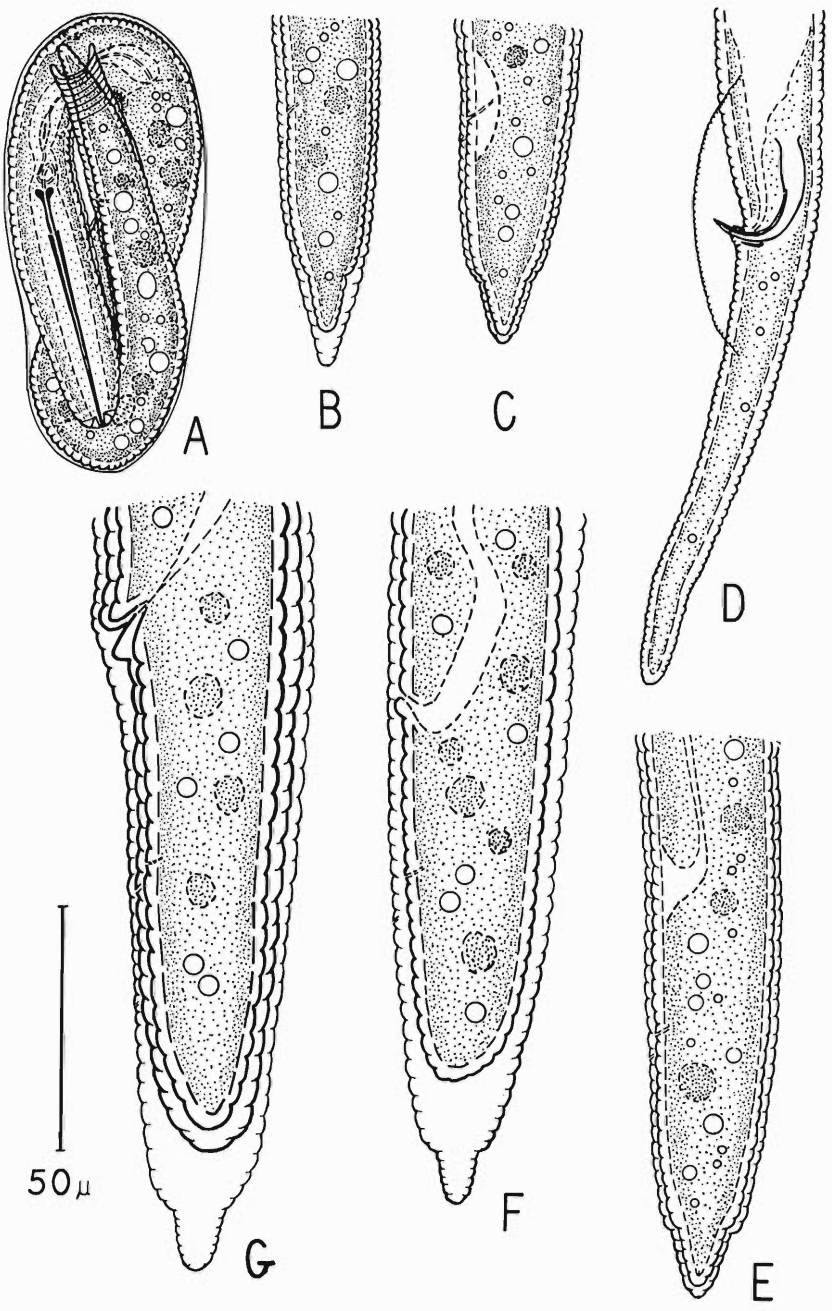

Figure 1. *Hemicycliophora arenaria* Raski. A. Second-stage larva within the egg. B. Tail of third-stage larva. C. Tail of fourth-stage male larva. D. Tail of adult male. E. Tail of fourth-stage female larva. F. Tail of young female within fourth-molt cuticle. G. Tail of adult female within fourth-molt cuticle.

There was a sixth cuticle produced by the adult female and not by the adult male. The extra cuticle on the female *Hemicycliophora* has been described as the "retention of the larval cuticle" by Colbran 1956, Luc 1958, Raski 1958, Tarjan 1952, and Thorne 1955 in all species of *Hemicycliophora* except *H. longicaudata* Loos and *H. straeleni* Coninek. In *H. arenaria* this is not a larval cuticle as we normally think of it, but an additional cuticle produced by the young female, representing an incomplete adult molt. This is in agreement with the illustrations of the above workers who described the "larval cuticle" as having an opening for the vulva and being attached at the vulva and head. None of the illustrations of adult females having an extra cuticle show remnants of a stylet between the cuticles, and surely this would have been illustrated had there been one. The species of *Hemicriconemoides* Chitwood, 1957, are a closely allied group of nematodes having a similar sheath on the adult female.

It appears that the production of a sixth cuticle probably occurs in nearly all the species of *Hemicycliophora* and in many of the species of *Hemicriconemoides*. To the taxonomist the sixth cuticle may be a useful generic character. If this is so, life history studies of other members of these genera may provide the solution to the existing confusion in the *Hemicycliophora-Criconemoides* complex.

SUMMARY

Hemicycliophora arenaria Raski has four larval molts, the first within the egg. Critical measurements are given for each stage. A period of feeding was necessary between each molt. The males became differentiated from the females during the fourth larval stage. The adult female produced a sixth cuticle prior to shedding the fourth larval cuticle so that the female nematode is ensheathed with two cuticles throughout its entire life. The life cycle from egg to egg required from 15-18 days at 28-30° C on tomato seedlings.

LITERATURE CITED

CHITWOOD, B. G. and W. BIRCHFIELD. 1957. A new genus, Hemicriconemoides (Criconematidae: Tylenchida). Proc. Helminth. Soc. Wash. 24: 80-86.

COLBRAN, R. C. 1956. Studies of plant and soil nematodes. I. Two new species from Queensland. Queensland J. Agric. Sci. 12: 123-126.

LUC, M. 1958. Trois nouvelles especes Africaines du genre *Hemicycliophora* de Man, 1921 (Nematoda: Criconematidae). Nematologica 3: 15-23.

PAETZOLD, D. 1958. Beobachtungen zur Stachellosigkeit der Mannchen von *Hemicycliophora typica* de Man 1921 (Criconematidae). Nematologica 3: 140-142.

RASKI, D. J. 1958. Four new species of *Hemicycliophora* de Man, 1921, with further observations on *H. brevis* Thorne, 1955 (Nematoda: Criconematidae). Proc. Helminth. Soc. Wash. 25: 125-131.

TARJAN, A. C. 1952. *The nematode genus Hemicycliophora* de Man, 1921. (Criconematidae) with a description of a new plant-parasitic species. Proc. Helminth. Soc. Wash. 19: 65-77.

THORNE, G. 1955. Fifteen new species of the genus *Hemicycliophora* with an emended description of *H. typica* de man (Tylenchida: Criconematidae). Proc. Helminth. Soc. Wash. 22: 1-16.

VAN GUNDY, S. D. 1957. The first report of a species of *Hemicycliophora* attacking citrus roots. Plant Disease Reptr. 41: 1016-1018.

VAN GUNDY, S. D. 1958. The pathogenicity of *Hemicycliophora arenaria* on citrus. Phytopathology 48: 399 (Abstr.).

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