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Proceedings of the Helminthological Society of Washington

VOLUME 34 JANUARY 1967 NUMBER 1

# Failure of Certain Clams and Oysters to Serve as Intermediate Hosts for *Angiostrongylus cantonensis*[1]

STUART E. KNAPP AND JOSEPH E. ALICATA

Several species of invertebrate animals have been reported as intermediate or as paratenic hosts for the rat lungworm, *Angiostrongylus cantonensis*. Intermediate hosts which have been found naturally infected include: the land snails, *Bradybaena similaris, Opeas javanicum, Macrochlamys resplendens, Achatina fulica, Pupina complanata,* and *Subulina octona*; the slugs, *Deroceras laeve, Vaginalus plebeius, Veronicella alte, Girasia peguensia,* and *Microparmarion malayanum*; and the amphibious snail, *Pila ampullacea*. Naturally infected paratenic hosts include: the land planarian, *Geoplana septemlineata*; the freshwater prawn, *Macrobrachium* sp.; the land crab, *Cardisoma hirtipes*; and the coconut crab, *Birgus latro*. Also, several land and aquatic snails and slugs have been reported as experimental hosts (Alicata, 1965).

Of particular interest have been the recent reports (Cheng and Burton, 1965 and Cheng, 1966) that the American oyster, *Crassostrea virginica*, and the soft-shell clam, *Mercenaria mercenaria*, could serve as intermediate hosts of *A. cantonensis* under experimental conditions. This finding could have special significance especially in some of the Pacific islands where the rat lungworm exists and clams and oysters may be eaten raw or imperfectly cooked.

In the present study, attempts were made to determine the following: (a) ability of local clams (*Venerupis philippinarum*) and oysters (*Crassostrea virginica*) to serve as intermediate hosts of *A. cantonensis*; (b) possible natural infection of certain species of clams and oysters in Hawaii and in the island of Ulong, Palau Islands, with third-stage larvae of *A. cantonensis*. These are areas in which murine angiostrongylosis is endemic.

## MATERIALS AND METHODS

Clams collected from Kaneohe Bay, Oahu, Hawaii, on 31 August 1965, were divided into 4 groups having 8 clams per group. Each group was placed in a glass aquarium containing 5,000 ml of continuously aerated sea water with a salinity of 15.1‰ and temperature of 20 ± 1 C. After 96 hr, the clams in the different aquaria were exposed to first-stage *A. cantonensis* larvae as follows: group 1, 38 larvae in 0.25 ml of water were injected into the mantle of each clam using a hypodermic syringe equipped with a 4-inch, 22-gauge needle which was inserted between the valves. The clams remained out of the water for 15 min after larval injection; group 2, 3,040 larvae were placed directly into the aquarium with the clams; group 3, similar to group 1 except that 76 larvae were injected into each clam; group 4, same as group 2 except that 6,080 larvae were placed in the water. No sand was used for a substrate so the clams rested on the glass bottom of their respective aquaria.

Oysters collected from Pearl Harbor, Oahu, Hawaii, on 1 February 1966, were divided into 6 groups having 5 oysters per group. Groups 1, 2, and 3 were placed in a glass aquarium containing 5 gallons of continuously

[1] Contribution from the Department of Veterinary Medicine, Oregon State University, Corvallis, and the Parasitology Laboratory, Department of Animal Sciences, University of Hawaii, Honolulu.

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The authors wish to acknowledge the assistance of Mr. Eugene Burke and Mr. Kenji Ego of the Hawaii Fish and Game Commission for assistance in obtaining clams and oysters from the island of Oahu. Mr. Peter Wilson, Fisheries Officer of the Trust Territory of the Pacific, supplied the clams from Ulong, Palau Islands. Mr. David Hashimoto of the U. S. Fish and Wildlife Service assisted in determining salinity of the sea water used in this study.

aerated sea water with a salinity of 15.3‰ and a temperature of 20 ± 1 C. Groups 4, 5, and 6 were placed in another similar aquarium with a salinity of 10.6‰. After 96 hr, the body of each oyster of groups 1 and 3 was injected with 0.02 ml normal saline solution containing approximately 5,000 first-stage larvae of *A. cantonensis*. Each injection was carried out by using a hypodermic syringe equipped with a 4-inch, 22-gauge needle which was carefully inserted between the valves. The oysters remained out of the water for 15 min after larval injection. For oysters of groups 2 and 5, the valves of each were slightly opened with a knife blade to drain the containing sea water, and then with the aid of a fine Pasteur pipette, 0.1 ml of saline solution containing approximately 10,000 first-stage lungworm larvae was ejected into the cavity between the two valves. In this operation, the oysters remained out of the water for 1 hr before they were replaced in their respective glass aquaria. Oysters of groups 3 and 6 were removed into two small glass aquaria, each containing 2,000 ml of continuously aerated sea water (salinity 15.3‰ and 10.6‰, respectively) to which approximately 150,000 freshly isolated first-stage rat lungworm larvae were added. After two days' exposure to larval infection, the two groups of oysters were replaced in their former larger aquaria.

All of the oysters were examined for larvae of *A. cantonensis* 25 days after injection or exposure to infection. This was carried out both by press preparation of parts of the tissues of each oyster, and by digesting the remaining parts of the oysters with the use of the pepsin–HCl digestion technique.

Oysters and clams which were collected and examined for natural infection with larvae of *A. cantonensis* included the following: 150 clams, *Venerupis philippinarum*, from Kaneohe Bay, Oahu, Hawaii; 50 clams, *Matra thaanumi*, from Ulong, Palau Islands; 100 oysters, *Crassostrea virginica*, from Pearl Harbor, Oahu, Hawaii. All these mollusks were examined by use of the pepsin–HCl digestion method.

## Results and Discussion

All the clams and oysters which were either injected or exposed to infection with first-stage larvae of *A. cantonensis* and examined by press preparation or artificial digestive methods 21 and 25 days later, respectively, failed to show either developing or infective third-stage larvae of the parasite. A few (1 to 2) dead and undeveloped first-stage larvae were recovered after artificial digestion of 3 clams from groups 3 and 4 which died 11, 12, and 17 days after exposure to larval infection. Similarly, 2 live and many dead and undeveloped first-stage larvae were found on press preparation of the tissue of an oyster (group 1) which died 4 days after injection with first-stage larvae.

It is concluded that under the conditions of these experiments, neither the clam, *V. philippinarum*, nor the oyster, *C. virginica*, serves as intermediate host of *A. cantonensis*. These data do not confirm the report of Cheng and Burton (1965) that *C. virginica* serves as an experimental host for this parasite. It is believed also that further tests are necessary to verify the reported claim that *M. mercenaria* can be infected with *A. cantonensis*. No natural infection with third-stage larvae of *A. cantonensis* was found among oysters or clams collected from the island of Oahu, Hawaii, nor from the island of Ulong, Palau Islands.

The failure either to infect or find natural infection of local clams and oysters with larvae of *A. cantonensis* suggests that these mollusks have little importance in the transmission of human angiostrongylosis in the Hawaiian Islands.

## Summary

An attempt was made to infect the Hawaiian edible clam, *Venerupis philippinarum*, and the American oyster, *Crassostrea virginica*, with first-stage larvae of *Angiostrongylus cantonensis*. The clams were maintained in glass aquaria containing sea water with a salinity of 15.1‰. The oysters were maintained in similar aquaria containing sea water with a salinity of 15.3‰ and 10.6‰. These mollusks were exposed to infection by either injecting their tissues with first-stage larvae of *A. cantonensis* or placing the larvae in the water. When these mollusks were examined by press preparation or artificial digestive methods, none of them was infected with third-stage larvae of the parasite. A few undeveloped and dead first-stage larvae were found in the tissues of 3 clams and one oyster which died a few days after exposure to infection.

No evidence of natural infection with larvae of *A. cantonensis* was found among 150 clams (*V. philippinarum*) and 100 oysters (*C. virginica*) collected from the shores of the island of Oahu, Hawaii, nor among 50 clams collected from the island of Ulong, Palau Islands.

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## *Myzotrema cyclepti* gen. n., sp. n. (Trematoda: Monogenea) from Gills of *Cycleptus elongatus* (LeSueur) from Alabama[1]

WILMER A. ROGERS[2]

The species described in this paper was collected as part of a survey of fish parasites being conducted by the Southeastern Cooperative Fish Parasite and Disease Project of the Agricultural Experiment Station, Auburn University. This species was collected using the 1:4,000 formalin field-collecting method described by Rogers (1966). Specimens were measured according to the procedure given by Mizelle and Klucka (1953). Measurements are expressed in microns and were made from specimens mounted in glycerin jelly or permount. Details of internal anatomy were determined from hematoxylin-stained specimens. Illustrations were prepared with the aid of a camera lucida. The keys to the genera of Ancyrocephalinae by Mizelle and Price (1964) and Yamaguti (1963) were useful in determining the status of the present species.

### *Myzotrema* gen. n.

GENERIC DIAGNOSIS: Dactylogyridae, Ancyrocephalinae: Body large, elongate, with two pairs of eyespots, head organs poorly developed or lacking. Opisthohaptor well set off from body proper by stout peduncle, with two pairs of nearly similar anchors, each pair supported by a nonarticulate transverse bar; 14 marginal hooklets present. Pharynx large, heavily muscularized, perfectly round in cross-section. Intestinal crura simple, united posteriorly. Testis and ovary equatorial, overlapping. Vas deferens looped around left intestinal crus, seminal vesicle formed by dilation of vas deferens. Two prostatic reservoirs present. Cirrus a U-shaped tube with complex accessory piece articulated to base. Ovary looping around right intestinal crus. Vagina present, opening dextroventrally; submedian or submarginal. Vitellaria coextensive with intestine. Parasitic on fresh water fish.

TYPE SPECIES: *Myzotrema cyclepti* sp. n.

TYPE HOST: Blue sucker, *Cycleptus elongatus* (LeSueur).

LOCALITY: Tombigbee River, Pickens County, Alabama.

REMARK: *Myzotrema* gen. n. is most closely related to *Pseudomurraytrema* Bychowsky, 1957 (nec *Pseudomurraytrema* Yamaguti, 1958) as shown by the structure of the copulatory complex and the reproductive system. It is readily separated from *Pseudomurraytrema* by

[1] Supported by the Southeastern Cooperative Fish Parasite and Disease Project.

[2] Agricultural Experiment Station, Auburn University, Auburn, Alabama.

having two instead of three haptoral bars. The copulatory complex of *Anoncohaptor* Mueller, 1938 (Mueller, 1938, plate 1, fig. 5) is also similar to that of the present genus, but *Anoncohaptor* has no haptoral anchors or bars. Both *Urocleidus* Mueller, 1934, and *Cleidodiscus* Mueller, 1934, have two pairs of anchors that are each supported by a transverse non-articulated bar (Yamaguti, 1963), but in neither genus does the vas deferens or ovary loop around the intestinal limbs as in Myzotrema.

*Myzotrema cyclepti* gen. n., sp. n.
(Figs. 1–8)

DESCRIPTION: A large form with elongate body and thin cuticle that may be finely striated. Length 1,885 (1,650 to 2,200), greatest width 301 (185 to 370) about midlength. Four eyespots present, approximately same size, posterior pair sometimes larger, members of posterior pair always farther apart. Head without lobes, head organs undeveloped or lacking. Pharynx perfectly round in cross-section, heavily muscularized, transverse diameter 131 (115 to 150). Intestinal limbs simple, uniting posteriorly. Peduncle long and stout; haptor obovate in outline with smooth margins, broader than long, length 147 (110 to 210), width 180 (170 to 210). One pair of dorsal and one pair of ventral anchors, similar in size and shape, each pair supported by a transverse bar. Each anchor composed of (1) a broad base without differentiated roots, (2) a solid short shaft grading into (3) a solid, short finely attenuated point. Anchor wings not observed. Ventral anchors (Fig. 4) often smaller than dorsal anchors; length 59 (57 to 64), width of base 36 (35 to 38). Dorsal anchor (Fig. 5) length 59 (53 to 64), width of base 38 (34 to 40). Two transverse nonarticulate bars present; ventral bar (Fig. 6) with prominent posteriorly projecting arch, dorsal bar (Fig. 7) approximately straight but with an anteriorly directed hump at midlength. Length of ventral bar 73 (72 to 75); length of dorsal bar 89 (84 to 96). Marginal hooks 14 in number, similar in size and shape (Fig. 3); small without inflated bases, length 16 (15 to 17). One hook pair is located ventrally in center of haptor while 3 pairs are located ventrally at anterior edge of haptor and 3 pairs are located dorsally in anterior one-third of haptor (Fig. 8). The median pair is situated immediately posterior to the ventral bar which according to Mizelle and Crane (1964) would be pair no. 5. Pairs 1, 2, and 3 would then be located ventrally while pairs 4, 6, and 7 would be located dorsally. Testis and ovary located slightly anterior to midlength; overlapping. Testis globose, located sinistrally in loop of ovary; vas deferens looping around left limb of intestine and running anteriorly, expands into seminal vesicle just posterior to cirrus. Copulatory complex composed of cirrus (Fig. 1) and accessory piece (Fig. 2). Cirrus a U-shaped tapering tube with an angular projection about midlength, with a complex basally articulating accessory piece. Two prostatic reservoirs empty into cirrus, one about twice as long as the other. Length of cirrus 50 (48 to 52); length of accessory piece 51 (47 to 55). Ovary vase-shaped with elongate neck folding over testis and looping around right intestinal limb. Vagina opening ventrally, submarginal or submedian; with a long coiled tube leading into a large pear-shaped seminal receptacle. Vitellaria coextensive with intestine, confluent over most of anterior two-thirds of body posterior to pharynx.

HOST AND LOCALITY: Blue sucker, *Cycleptus elongatus* (LeSueur), Tombigbee River, Pickens County, Alabama.

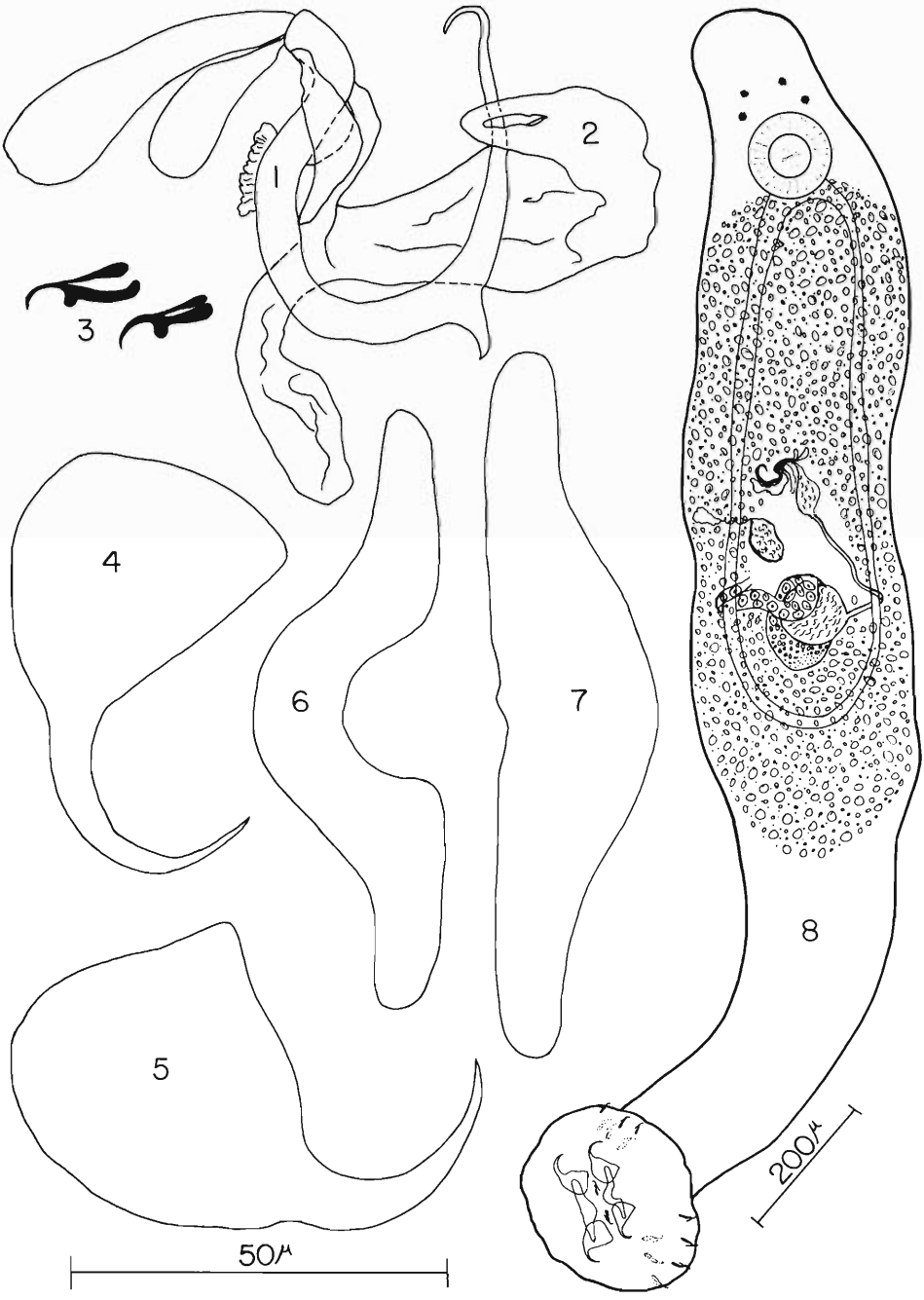
SPECIMENS STUDIED: Eight.

TYPE SPECIMENS: Type, USNM Helm. Coll. No. 61248; 1 paratype, USNM Helm. Coll. No. 61249; paratypes in author's collection.

REMARKS: *Myzotrema cyclepti* is the only described species in this genus. The nature of the copulatory complex, vagina, ovary, and other reproductive structures is very similar to those observed in *Pseudomurraytrema* species (Rogers, 1966); *P. copulatum* (Mueller) and *Anoncohaptor anomalus* Mueller (Mueller, 1938).

ACKNOWLEDGMENTS

Thanks are extended to William Smith-Vaniz, Division of Fisheries, Smithsonian Institution, Washington, D. C., and to Walter Tatum and other members of the Alabama Department of Conservation for assistance in collecting the fish hosts.




Figs. 1–8. *Myzotrema cyclepti* gen. n., sp. n. 1. Cirrus; 2. accessory piece; 3. marginal hooks; 4. ventral anchor; 5. dorsal anchor; 6. ventral bar; 7. dorsal bar; 8. entire worm, ventral aspect.

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## A Review of the Genus *Pseudocella* Filipjev, 1927 (Nematoda: Leptosomatidae) with a Description of *Pseudocella triaulolaimus* n. sp.

W. DUANE HOPE[1]

The genus *Thoracostoma* Marion, 1870 was separated into the subgenera *Thoracostoma* and *Pseudocella* by Filipjev (1927), the former subgenus to receive those species with lens-bearing ocelli, symmetrical, but irregularly curved spicula, and gubernacula with apophyses directed dorsally and parallel with the spicula, and the latter subgenus to receive those species with ocellar pigment spots lacking a lens, spicula often asymmetrical but uniformly curved, and gubernacula with paired apophyses directed posteriorly at right angles to the spicula. Later, Filipjev (1946) raised both subgenera to generic rank.

Wieser (1953) continued to treat Filipjev's taxa as subgenera of the genus *Thoracostoma* and later added two additional subgenera, *Corythostoma* and *Synonchoides* (Wieser, 1956). *Corythostoma* was proposed to receive *T. kreisi* Wieser, 1953 and *T. filipjev* Kreis, 1928 which resemble species of *Pseudocella*, but which possess a cephalic capsule with weakly developed posterior lobes separated by notches instead of incisures and which lack incisions or fenestrae, with amphids totally, or with their greatest portion, behind the posterior margin of the cephalic capsule, and ocelli absent. *Synonchoides* received a single species, *T. galathea* (Wieser, 1956) which also resembles species of *Pseudocella*, but which possesses a cephalic capsule reduced to a ring with a slightly notched posterior edge, no anterior lobes, with amphids completely posterior to the lobes and a buccal armature resembling that of *Synonchus* Cobb, 1893. From the two male specimens of *T. galathea*, Wieser was unable to determine if the gubernaculum possessed caudally directed apophyses.

Platonova (1962) ranked *Thoracostoma* and *Pseudocella* as genera and assigned *T. kreisi* and *T. filipjevi* to *Pseudocella* without reference to Wieser's subgenera.

The differences between *Thoracostoma* and *Pseudocella* have remained much the same even though new species have been added to both groups. A notable exception, as pointed out by Wieser (1956), is that species of *Thoracostoma* have been described which do not possess ocellar pigment spots or lenses (Wieser, 1956; Allgen, 1951; Schuurmans Stekhoven and Mawson, 1955). Consequently, emphasis has shifted from differences in the ocelli to differences in the spicula and gubernacula. The fact remains, however, that there are

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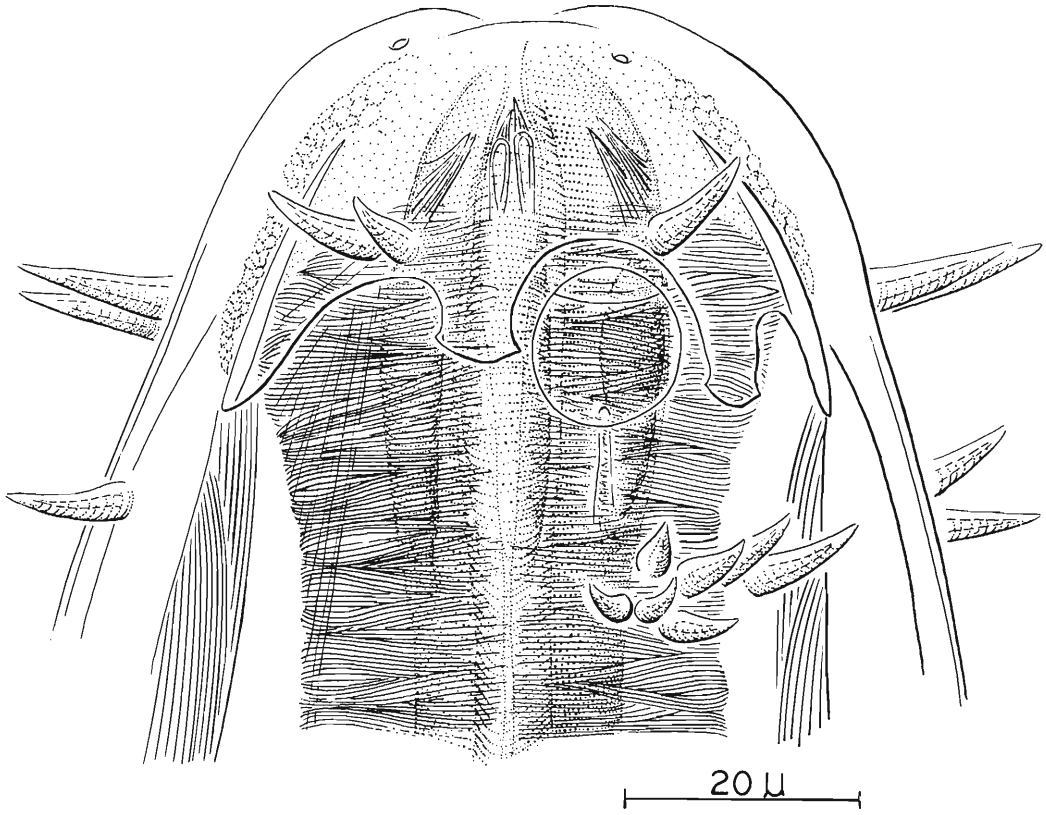


Fig. 1. *Pseudocella triaulolaimus* n. sp. Head of holotype in lateral view. Dorsal and subventral interradial teeth not illustrated.

relatively distinct and, at present, consistent morphological differences between these two groups and, for these reasons, their generic status is herein retained.

Species of *Corythostoma* differ from those of *Thoracostoma* and *Pseudocella* in the structure of the cephalic capsule, as mentioned above, and for this reason, retaining *Corythostoma* as a subgenus can be justified, pending further study. On the other hand, it is difficult to justify raising *Corythostoma* to the generic level since the males of this group, that have been adequately studied, have spicula and gubernacula identical to those of *Pseudocella*. Therefore, the subgenus *Corythostoma* is here transferred from *Thoracostoma* to the genus *Pseudocella*.

It is also here proposed that *Synonchoides* be provisionally considered a subgenus of *Pseudocella*. A more satisfactory arrangement must be based on additional studies of the stomatal armature, spicula, and gubernaculum of *P.* (*Synonchoides*) *galathea* (new combination). The taxonomic status of this species might also be clarified by a detailed study of the stomatal armature in species of the genus *Synonchus*.

All species of the genus *Pseudocella* not here assigned to the subgenera *Corythostoma* and *Synonchoides* are regarded as members of the nominate subgenus *Pseudocella*, which has the posterior lobes of the cephalic capsule separated by notches; a claviform piece present; amphids completely surrounded by lobes of the cephalic capsule; ocelli absent; spicula of uniform diameter and regularly curved; and

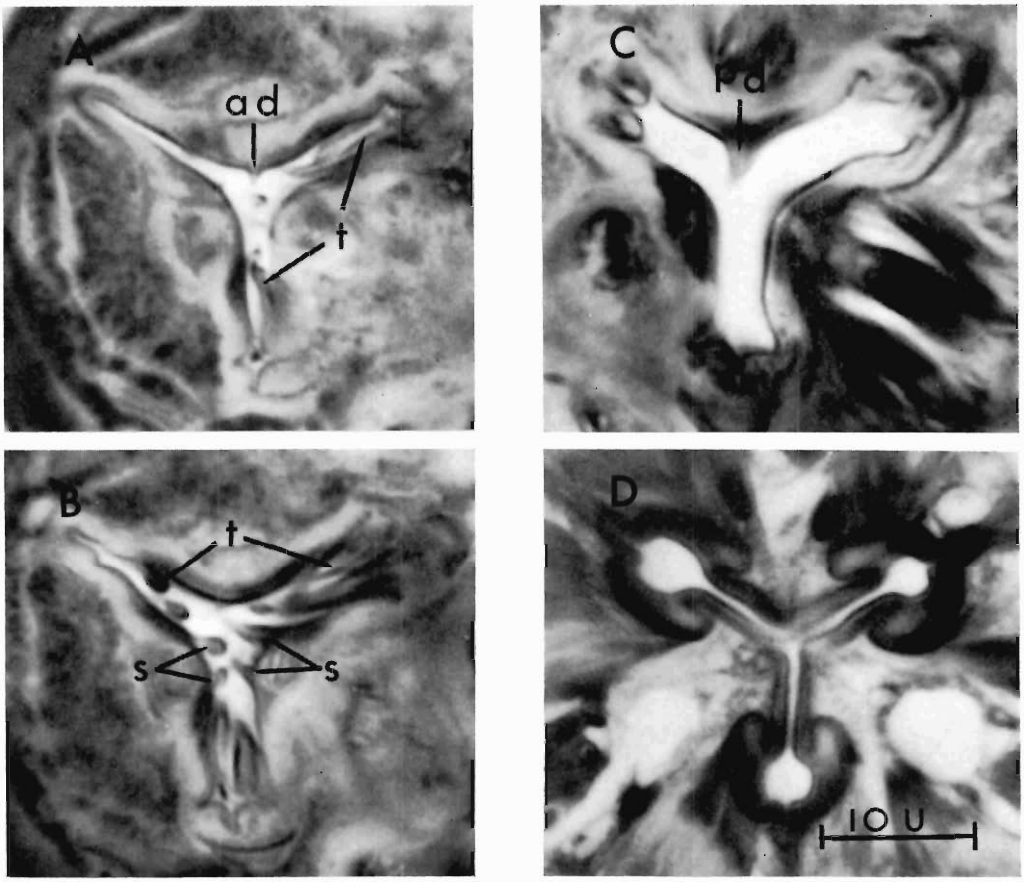


Fig. 2. *Pseudocella triaulolaimus* n. sp. Photomicrographs of cross-sections through stoma. Sections A through D approximately 5, 10, 15, and 30 $\mu$, respectively, from anterior extremity. Anterior dorsal interradial tooth (ad); teeth of radii (t); subventral interradial teeth (s); posterior dorsal interradial tooth (pd).

gubernacula with caudally directed apophyses (Wieser, 1956).

### *Pseudocella* (*Corythostoma*) *triaulolaimus* n. sp.

Sixteen male, three female, and three juvenile specimens of *Pseudocella triaulolaimus* n. sp. were collected in February, 1963 from sandy sediment held by holdfasts of *Egregia menziesii* (Turner). The holdfasts were attached to rocks in the intertidal zone at Dillon Beach, California. The nematodes were relaxed for 30 minutes in sea water saturated with carbon dioxide, fixed in 10% formalin in sea water and mounted in glycerine or CMC10*. The latter was used principally for males, for the specimens are rendered more transparent in this mounting medium and a detailed study of the stomatal structure and male genital apparatus is made possible. The head of one male was mounted *en face* in glycerine jelly and one male and one female were embedded in polyethylene glycol and sectioned at 5 $\mu$. The sections were stained with hematoxylin at pH 2.4 by the method of Craig and Wilson (1937).

* Product of Turtox Biological Supply House Inc.

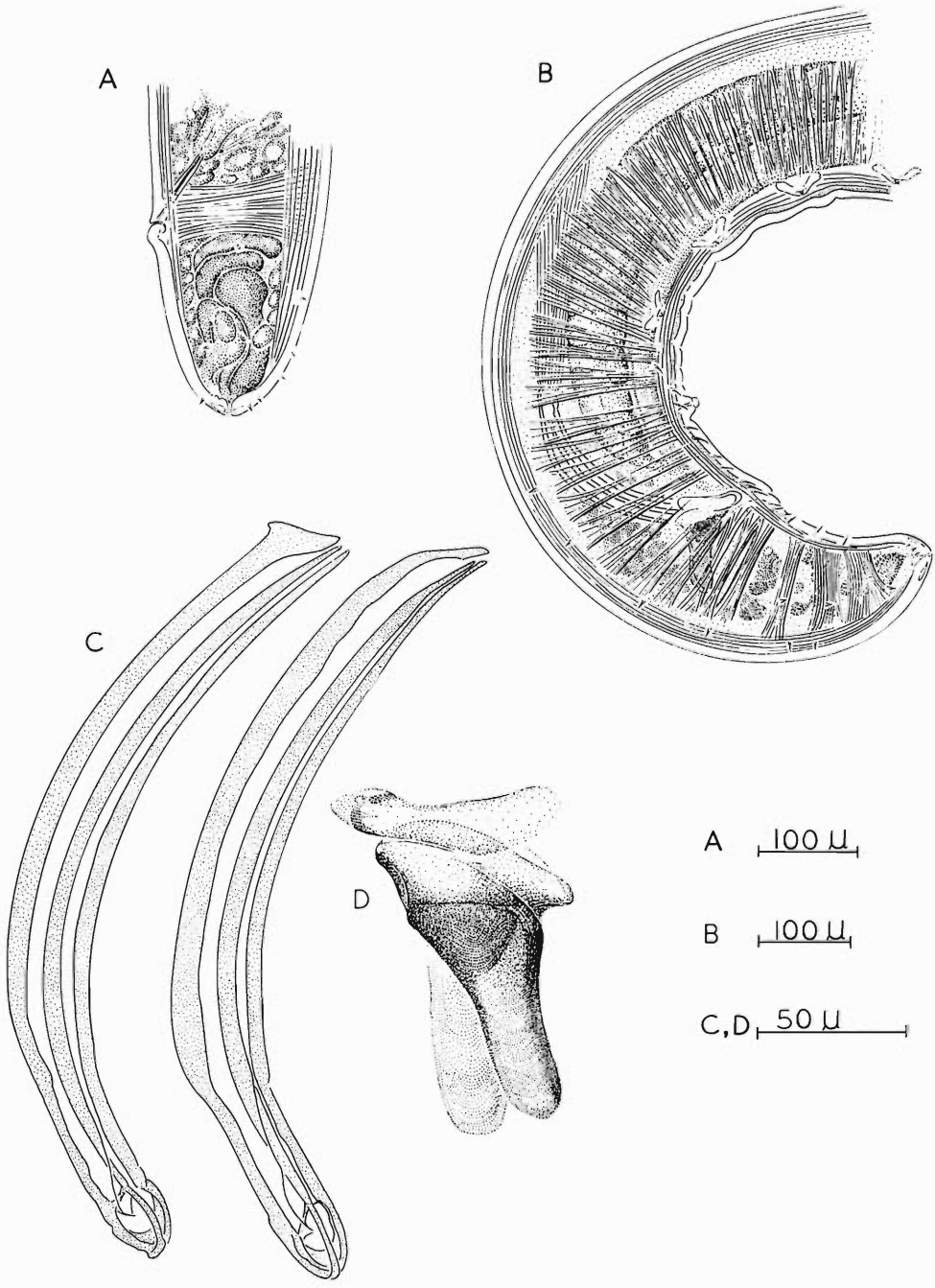


Fig. 3. *Pseudocella triaulolaimus* n. sp. (A) Tail of allotype (female). (B) Tail of holotype (male). (C) Left (with distal hook) and right spicula of male paratype. (D) Gubernaculum of male paratype.

MEASUREMENTS

HOLOTYPE (Male): L = 16.80 mm; a = 74.7; b = 8.27; c = 69.13; T = 45%.

ALLOTYPE (Female): L = 19.72 mm; a = 88.4; b = 8.80; c = 93.0; V = (24.6%) 62.9% (22.6%).

PARATYPES (13 males): L = 14.53–20.89 mm (18.75); a = 51.5–83.5 (72.23); b = 7.27–13.47 (9.19); c = 60.04–88.60 (73.09); T = 45.0%–71.6% (60.9%).

PARATYPE (1 female): L = 18.09 mm; a = 58.7; b = 8.89; c = 88.7; V = (14.8%) 64% (18.0%).

PARATYPES (3 juveniles): L = 12.06–13.96 (13.15); a = 50.7–72.7 (62.6); b = 6.03–8.31 (7.46); c = 58.1–73.9 (6.77).

DESCRIPTION: Body long, slender, and tapering anteriorly from base of esophagus; posterior extremity tapering from short distance anterior to cloaca. Integument without transverse or longitudinal striations; cuticle 10.5–6.1 (8.69) $\mu$ thick at mid-body level. Six papillae on lips followed by circle of ten cephalic setae, the latter, 11.0 to 17.5 $\mu$ long.

Cephalic capsule with well-developed posterior lobes; sublateral lobes only partially enclosing amphids; lobes separated by deep notches and lacunae absent. Anterior lobes short; claviform piece absent. Major portion of cephalic capsule separated from integumentary cuticle by alveolar substance (tissue?). Amphids circular, ⅕ width of corresponding head diameter and situated just posterior to lateral cephalic setae.

Cervical setae between cephalic capsule and nerve ring in dorsal, lateral, and ventral longitudinal rows. These setae long (11.0 to 17.0 $\mu$), numerous and in clusters near posterior margin of cephalic capsule, becoming sparse and shorter (3.0 to 5.0 $\mu$) at level of nerve ring. Setae posterior to nerve ring 2.0 to 3.0 $\mu$ long and sparsely distributed.

Stoma triradiate in cross-section with 12 teeth, two at distal end of each radius (Fig. 2A and B; t) and two on each of three interradial facets; subventral interradial teeth juxtapose (Fig. 2B; s) and dorsal interradial teeth tandem; anterior dorsal tooth 1.0 $\mu$ long (Fig. 2A; ad), posterior dorsal tooth 3.5 $\mu$ long (Fig. 2C; pd); all teeth directed anteromedially. Distal ends of radii tuboid and walls of radii greatly thickened for a distance of approximately 30 $\mu$ posterior to teeth (Fig. 2D). Further posteriorly, distal ends of radii convergent, walls thin but with muscle attachment points and muscles concentered.

Esophagus muscular and gradually increasing in diameter towards its posterior end. Nerve ring located approximately ⅓ the length of the esophagus from the anterior end. Renette cell and ocellar pigment spots absent.

MALES: Longitudinal series of subventral setae extending from near mid-region of tail to posterior subventral supplements; 16–22 in right subventral series, 17–21 in left subventral series; setae 15–25 $\mu$ long. Four to six setae distributed irregularly around caudal gland pore. Heavily cuticularized, hemispherical copulatory supplements in one subventral row on each side of body anterior to anus; four to six supplements on right side, four to seven on the left side; position of supplements relative to those of the opposite side either even or staggered; each supplement bearing a minute apical papilla.

Reproductive system diorchic, testes opposed and outstretched. Spicula (Fig. 3C) rather uniformly arcuate and comprised of three longitudinal ribs; proximal end of spicula devoid of dilated capitulum; distal end of left spiculum thickened, with a pointed posterior process. Right spicula 292.6–319.5 $\mu$, left spicula 270.7–324.4 $\mu$ long. Corpus of gubernaculum (Fig. 3D) with thin-walled processes embracing spicula laterally and with heavily cuticularized apophyses extending at right angles to spicula in a dorso-caudal direction; apophyses fused at their bases by a medial triangular thickening. Paired trumpet-shaped structures (Fig. 3D), ventro-lateral to distal end of spicula, apparently fused laterally with gubernaculum; these structures with a small pore near their distal end.

Posterior end of body strongly curved toward venter. Copulatory muscles well developed in approximately the posterior 11% of the body length. Tail conical with terminal gland pore; caudal glands convoluted, not extending anterior to gubernaculum.

FEMALES: Vulva without distinctly protruding lips. One to three rounded cuticular elevations anterior and posterior to vulva on ventromedial body surface (Fig. 4); setae not present on these elevations. Five to six hypo-

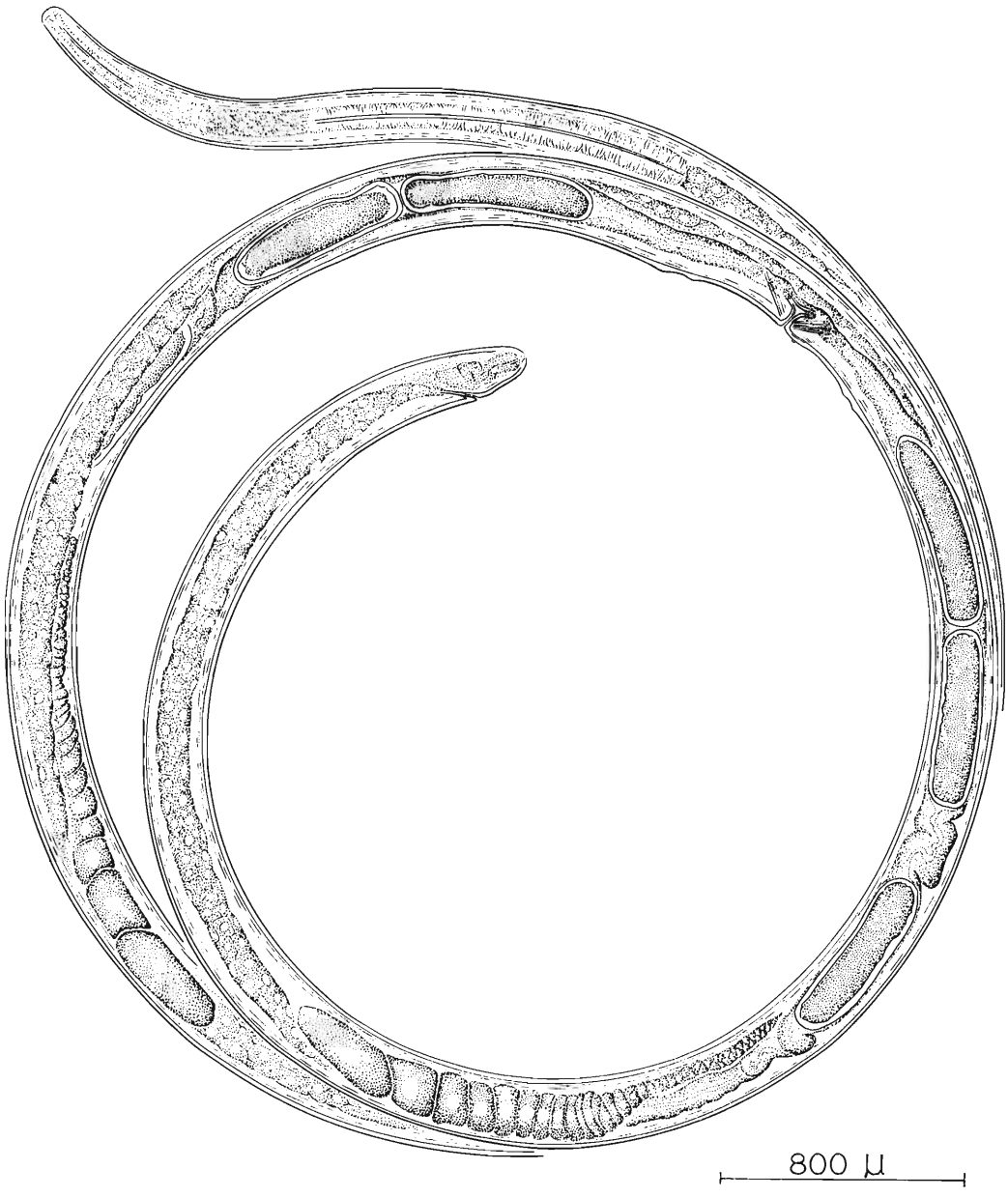


Fig. 4. *Pseudocella triaulolaimus* n. sp. Allotype (female).

dermal gland cells in longitudinal series in each lateral hypodermal chord at level of vulva; gland cells opening to the exterior by minute pores on ventral margin of each lateral chord.

Reproductive system amphidelphic and reflexed. Ova with chorion 560–653 (601) $\mu$ long and 127–147 (136.5) $\mu$ wide. Tail (Fig. 3A) conical, terminus bluntly rounded.

HOLOTYPE (Male): United States National Museum catalogue number 33631.

ALLOTYPE (Female): United States National Museum catalogue number 33632.

PARATYPES (Males, female, and juveniles): United States National Museum catalogue numbers 33633–33650.

TYPE HABITAT: Sediment held by the holdfasts of *Egregia menziesii* (Turner) attached to intertidal rocks.

TYPE LOCALITY: Dillon Beach, California.

REMARKS: *Pseudocella triaulolaimus* n. sp. has been assigned to this genus because of the presence of a cephalic capsule, a relatively short, bluntly rounded tail, uniformly curved spicula and gubernacula with caudally directed apophyses; to the subgenus *Corythostoma* because the lobes of the cephalic capsule are reduced in length and separated by notches only, neither incisions or fenestrae are present and amphids have at least half their length situated posterior to the caudal margin of the lobes.

It might be argued that *P. triaulolaimus* belongs in the subgenus *Synonchoides* because of the multiple teeth in the stoma. It is not assigned to this subgenus since the cephalic capsule is not reduced to a narrow band and because it is difficult at this time to appraise the apparent similarities of the stomatal armature without knowing the exact structure and location of teeth present in *P.* (*S.*) *galathea*.

*Pseudocella triaulolaimus* n. sp. can be distinguished from all species of *Pseudocella* by the tuboid radii and thickened walls in the posterior portion of the stoma and by the greater number of stomatal teeth.

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# Incidence of Bovine Coccidia in Western Oregon[1]

PETER A. NYBERG, DONALD H. HELFER, AND STUART E. KNAPP

Eleven or more species of coccidia of the genus *Eimeria* are currently recognized as occurring in bovine animals. At least 9 species are known to occur in this country, their incidence having been determined through field surveys of cattle conducted in various parts of the United States, particularly the Southeastern, North-Central, and Western states (Christensen, 1941; Davis, *et al.*, 1955; Hasche and Todd, 1959; Fitzgerald, 1962). These species include *E. bovis*, *E. zurni*, *E. ellipsoidalis*, *E. auburnensis*, *E. cylindrica*, *E. subspherica*, *E. canadensis*, *E. bukidnonensis*, and *E. alabamensis*. Two species, *E. bovis* and *E. zurni*, are consistent pathogens and cause acute coccidiosis (Hammond, *et al.*, 1944; Boughton, 1945; Davis and Bowman, 1951). *Eimeria ellipsoidalis* and *E. alabamensis* may produce no observable symptoms unless large numbers of sporulated oocysts are ingested or unless the calf is weakened from other predisposing conditions (Davis and Bowman, 1956). *Eimeria cylindrica* was reported by Wilson (1931) (as cited by Levine, 1961, p. 172), to be moderately pathogenic while *E. auburnensis*, *E. bukidnonensis*, and *E. canadensis* have a relatively low degree of pathogenicity (Hammond, *et al.*, 1961; Levine, 1961). The pathogenesis of *E. subspherica* is unknown.

Although bovine coccidia have been found in Pacific Northwest livestock, no incidence report for the various species has been made. It was for this reason that the present study was undertaken.

## MATERIALS AND METHODS

Tared sample bottles containing 2.5% potassium dichromate were distributed among three veterinary offices in Tillamook County, Oregon. This geographic location was selected because of the concentrated dairy industry there, and because it has climatic conditions representative of other Pacific Northwest dairy areas. Practitioners were asked to randomly collect fecal samples from two-week to one-year-old dairy calves between August and November, 1965. They were also asked to record the animal's sex, age, and fecal consistency for each sample submitted.

The approximate number of oocysts per gram of feces was determined using a modified McMaster method (Whitlock, 1948). After weighing, contents of each tared sample bottle were emptied into a beaker and water added to make a total volume of 100 ml. Following thorough mixing, a 1.0 ml aliquot (1/100 of original sample) was combined with 2.0 ml Sheather's sugar solution. After vigorous shaking, the oocysts in 1/20 (0.15 ml) of this 3.0 ml mixture were counted, using a McMaster counting chamber (representing 1/2,000 of the fecal material in the original sample).

## RESULTS AND DISCUSSION

### *Incidence and identification of species*

Of a total of 86 fecal samples examined, 62 (72%) contained oocysts of either a single or a mixture of species. From these samples, 8 species of coccidia were identified (Christensen and Porter, 1939; Christensen, 1941; Nyberg and Hammond, 1965). In order of their decreasing frequency, they were: *Eimeria bovis*, *E. ellipsoidalis*, *E. zurni*, *E. auburnensis*, *E. cylindrica*, *E. subspherica*, *E. bukidnonensis*, and *E. alabamensis*.

Table 1 shows the majority of positive samples contained mixtures of the various species; however, *E. bovis*, *E. ellipsoidalis*, *E. auburnensis*, *E. zurni*, *E. cylindrica*, and *E. bukidnonensis* were found occurring as single infections, representing 28% of the total samples.

### *Discharge of oocysts*

The number of oocysts per gram of feces ranged from 500 to over 10 million for the various species present. The range for the

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The authors wish to acknowledge the cooperation of Drs. Roy H. Peterson and LeRoy V. Gallagher, Tillamook; Dr. Ralph H. Perkins, Nehalem; and Dr. Kenneth J. Gallagher, Cloverdale, for their assistance in obtaining fecal samples used in this study.

TABLE 1. Incidence of bovine coccidia in 86 dairy calves from Western Oregon.

| Species of coccidia | Number of occurrences: | | |
|---|---|---|---|
| | Total | Single | Mixed |
| *E. bovis* | 53 (62%) | 18 (21%) | 35 (41%) |
| *E. ellipsoidalis* | 28 (33%) | 2 ( 2%) | 26 (31%) |
| *E. zurni* | 20 (23%) | 1 ( 1%) | 19 (22%) |
| *E. auburnensis* | 12 (14%) | 2 ( 2%) | 10 (12%) |
| *E. cylindrica* | 9 (10%) | 1 ( 1%) | 8 ( 9%) |
| *E. subspherica* | 7 ( 8%) | 0 ( 0%) | 7 ( 8%) |
| *E. bukidnonensis* | 5 ( 6%) | 1 ( 1%) | 4 ( 5%) |
| *E. alabamensis* | 1 ( 1%) | 0 ( 0%) | 1 ( 1%) |

two most pathogenic species (*E. bovis* and *E. zurni*) was from 425 to 2 million, and 370 to 8 million, respectively. A previous report (Horton-Smith, 1958), stated that a discharge rate in excess of 5,000 oocysts per gram is indicative of clinical coccidiosis. We found 36 of the 62 positive animals (58%) passing 5,000 or more oocysts of *E. bovis* or *E. zurni*, per gram of feces. Of these, 21 (59%) had normal stools, 9 (25%) were loose or scouring, and no history was given for 6 animals.

Twenty of 22 negative animals, for which the age, sex, and consistency of feces was known, were 4 months old or older. Perhaps this finding is related to the immunity observed in older animals that have been previously exposed to bovine coccidia.

## SUMMARY

Sixty-two (72%) of 86 fecal samples collected randomly from dairy calves in Tillamook County, Oregon, contained oocysts of the genus *Eimeria*. Eight species were identified. Listed in order of their decreasing frequency, they were: *Eimeria bovis* (62%), *E. ellipsoidalis* (33%), *E. zurni* (23%), *E. auburnensis* (14%), *E. cylindrica* (10%), *E. subspherica* (8%), *E. bukidnonensis* (6%), and *E. alabamensis* (1%). Twenty-eight per cent of the 86 samples contained single species, of which *E. bovis* comprised 21%. All species except *E. subspherica* and *E. alabamensis* occurred singly.

Oocyst discharge, involving all samples, ranged from 500 to over 10 million per gram of feces. Fifty-eight per cent of the animals harboring *E. bovis* and *E. zurni* were passing 5,000 oocysts or more per gram of feces, however, only 25% were scouring.

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# *Plicatobothrium cypseluri* n. gen., n. sp. (Cestoda: Pseudophyllidea) from the Caribbean Flying Fish, *Cypselurus bahiensis* (Ranzani, 1842)

R. M. CABLE AND MARY B. MICHAELIS[1]

The adult helminths found in 185 species of marine teleosts examined in Curaçao and Jamaica in 1961 included 178 species of digenetic trematodes and seven of acanthocephala, but only two species of cestodes were recovered. Both were pseudophyllideans. Although the scolex was not found for a specimen from the houndfish, *Strongylura timucu* (Walbaum, 1792), examined in Jamaica, the strobila identified the cestode as a species of *Ptychobothrium* Loennberg, 1889, possibly *P. belones* (Dujardin, 1845). Cestodes referred to that species have been reported from houndfishes in widely separated localities. Four complete specimens of the second cestode were removed from the intestine of flying fish in Curaçao, fixed in corrosive sublimate-acetic acid, and preserved for study later. Three were prepared as whole mounts stained with Harris' hematoxylin or Semichon's carmine followed by indulin to bring out surface features. The scolex of the fourth specimen was used for cross-sections and portions of the strobila for serial sections cut in the transverse and frontal planes. Drawings were made by microprojection and measurements are in millimeters.

Because features of the species from flying fish exclude it from any existing genus, a new one is erected to receive it and is characterized as follows:

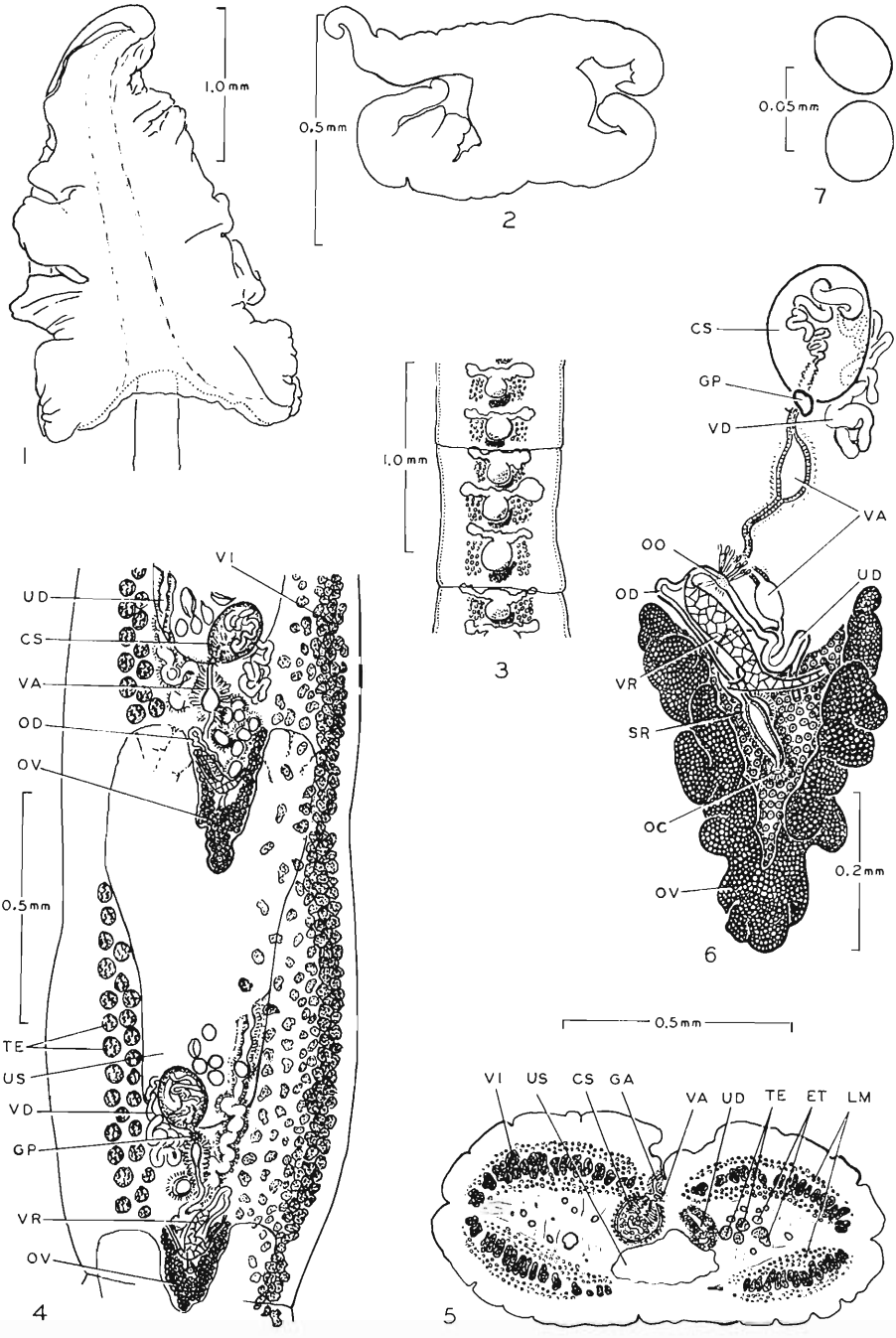
## *Plicatobothrium* n. gen.

Order Pseudophyllidea, Family Ptychobothriidae. Scolex basically H-shaped in cross-section, triangular to fan-shaped from lateral aspect, without apical organ or hooks. Bothria two, deep, open their entire length, not connected at tip of scolex; their walls extensively convoluted and folded, lined with minute, hair-like spines. Neck very short if present; strobila acraspedote, distinctly segmented only at intervals. Genital pore dorsal, median, posterior to midlevel of proglottid. Uterine pore midventral, inconspicuous, at posterior end of large, Y-shaped uterine sac with thin wall. Ovary at extreme posterior end of proglottid, flanked by arms of uterine sac in following proglottid. Uterine duct long, extensively coiled in zone between ovary and genital pore, then extending anteriorly to enter stem of uterine sac dorsolaterally at a distance from its anterior end. Vitelline follicles densest laterally but encompass medullary parenchyma except in vicinity of reproductive pores; follicles between conspicuous layers of longitudinal muscle fibers that are not grouped into distinct bundles. Testes in lateral regions of medullary parenchyma. Cirrus sac without external prostatic cells. Eggs thin-shelled non-operculate. Adults in intestine of marine fish, life history unknown. Type and only species:

### *Plicatobothrium cypseluri* n. gen., n. sp. (Figures 1–7)

DESCRIPTION (based on four mature specimens): with the characters of the genus. Scolex disproportionately large in comparison with strobila, triangular from lateral aspect of fixed specimens, 2.5–2.9 long, 1.3–1.8 in maximum width; its shape in living specimens continuously changed by movements of extremely active, muscular bothria which overhang junction of strobila and scolex. Strobila about 50.0 long; proglottids wider than long until gravid, then becoming much longer than wide. Primordia of reproductive organs evident a short distance from scolex. Uterine sacs develop in immature proglottids, first the more dorsal stem as a spherical cavity, then sacculations that become the arms; sacs Y-shaped with short arms in extended proglottids, cordiform when distended in contracted ones; uterine pore median, slightly posterior to genital pore on opposite side of proglottid. Vitelline reser-

[1] Department of Biological Sciences, Purdue University, Lafayette, Indiana. Specimens serving as the basis of this paper were collected in 1961 as part of an investigation then supported by Grant G-14691, National Science Foundation. Laboratory facilities were kindly extended by the Caribbean Marine Biological Institute, Curaçao, N.A., and by the Marine Biological Laboratory, University College of the West Indies, Jamaica, W.I.



voir dorsal, conspicuous, always tilted to side on which uterine duct enters uterine sac: usually on left but irregularly alternating; cirrus sac always tilted from median line to opposite side. Ovary median, triangular in outline but appearing broadly chevron- to V-shaped in whole mounts, depending on contraction, because of greater thickness at sides where lobules are dorsal to thinner connecting portion. Oocytes collect in that portion; oviduct begins there with oocapt, followed by enlarged, muscular segment, joined distally by small spherical enlargement at end of vagina. From that junction, oviduct extends as a narrow tube to end of vitelline reservoir where it turns, receives vitelline duct, and joins ootype well to side of median line. At ootype, uterine duct begins as a thin-walled tube which forms several loops, then becomes heavily muscular and much convoluted before extending anteriorly to enter uterine sac. From its enlargement at junction with oviduct, vagina also begins as a sinous, thin-walled tube, often with several large dilatations, then becomes heavily muscular before extending anteriorly to open into genital atrium; a short distance from that opening, vagina often with conspicuous enlargement sometimes containing oocytes as well as sperms. Testes scattered in lateral medullary parenchyma from ovary to bifurcation of uterine sac. Cirrus sac oval to pyriform, measuring 0.128–0.155 by 0.10–0.122 in mature proglottids, its length increasing to 0.162–0.182 in terminal portion of strobila in which uterine sacs are dilated but almost empty. Eggs oval, non-collapsed ones in whole mounts 0.045–0.048 long, 0.031–0.037 wide; none develop in uterus sufficiently to show larval hooks.

HOST: *Cypselurus bahiensis* (Ranzani, 1842).

LOCALITY: Curaçao, Netherlands Antilles.

TYPE SPECIMEN: Holotype No. 61346, Helm. Coll., USNM.

Features of *Plicatobothrium cypseluri* place it in the Family Ptychobothriidae Lühe, 1902, as recognized by Wardle and McLeod (1952) and Yamaguti (1959). It seems closest to *Ptychobothrium belones*, the only described species of *Ptychobothrium* which, like *Plicatobothrium*, has a scolex that bears only two deep bothria, open their entire length and not connected anteriorly. Further indication of their affinity is suggested by the fact that synentognath fishes serve as hosts of both species. In other respects, however, they show differences that justify their allocation to separate genera. Unlike *Plicatobothrium*, *Ptychobothrium* has the genital pore anterior to the midlevel of the proglottid, and a uterine duct that extends from the female complex in a wide curve to the right or left, to cross the anterior end of the proglottid and join a small, spherical uterine sac which opens at a conspicuous pore far to one side of the median line. Moreover, eggs develop embryos with hooks and are stored largely if not entirely in the uterine duct which consequently becomes distended with eggs and forms convolutions that fill most of the medullary parenchyma in gravid segments.

Although *Ptychobothrium* and *Plicatobothrium* are at present monotypic genera, it seems likely that further and more precise studies will reveal additional species. During his studies in the Galapagos Islands, Manter (personal communication) found in a flying fish a scolex resembling that of *P. cypseluri* but of a somewhat different shape. Even greater differences have been described for scoleces of cestodes that have been obtained from houndfishes and identified as *P. belones*.

## SUMMARY

A species of *Ptychobothrium* was recovered from the intestine of the houndfish, *Strongy-*

←

Figs. 1–7. *Plicatobothrium cypseluri*. 1. Scolex in lateral aspect. 2. Cross-section of scolex near tip. 3. Immature portion of strobila showing external segmentation at intervals and early appearance of uterine sacs. 4. Proglottid near end of strobila. 5. Cross-section of mature proglottid at level of genital pore and a short distance anterior to uterine pore. 6. Reproductive complex in proglottid near end of strobila. 7. Eggs.

Abbreviations: CS, cirrus sac; ET, excretory tubules; GP, genital pore; LM, longitudinal muscles; OC, oocapt; OD, oviduct; OO, ootype; OV, ovary; SR, seminal receptacle; TE, testes; UD, uterine duct; US, uterine sac; VA, vagina; VD, vas deferens; VI, vitellaria; VR, vitelline reservoir.

*lura timucu*, in Jamaica, and *Plicatobothrium cypseluri* n. gen., n. sp. is described from the flying fish, *Cypselurus bahiensis*, in Curaçao. *Plicatobothrium* and *Ptychobothrium* have similar scoleces but are differentiated by large, Y-shaped uterine sacs opening posteriorly, genital pores posterior to the midlevel of the proglottids, collection of eggs largely in the uterine sac, and their discharge before embryos develop hooks in *Plicatobothrium*.

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## Morphological Differences Between the Cuticle of Swarming and Nonswarming *Tylenchorhynchus martini*

I. K. A. IBRAHIM*

The phenomenon of swarming in *Tylenchorhynchus martini*, Fielding, 1956, was first reported by Hollis (1958), and several reports were published subsequently on its induction and nature (Hollis, 1960, 1962; Hollis and McBride, 1962; McBride, 1964; McBride and Hollis, 1966). Results of the present study indicate that there are morphological differences between the cuticle of swarming and nonswarming populations of *T. martini*. Such differences appear in swarming specimens as morphological changes in some cuticular layers, especially the cortex and the fiber layers. These morphological changes do not appear in the cuticle of nonswarming specimens of *T. martini*. The results also indicate that the cuticle of *T. martini* is a multilayered structure similar to that of *Meloidogyne javanica* (Bird and Rogers, 1965) and *Xiphinema index* (Wright, 1965).

### MATERIALS AND METHODS

Swarming and nonswarming specimens of *T. martini*, grown on rice plants in the greenhouse, were extracted from the soil and placed in 1% unbuffered osmium tetroxide solution for 2 hr at 5 C. Specimens were then washed in distilled water and dehydrated gradually in a series of ethanol–water solutions, followed by incubation in the following: absolute ethanol, propylene oxide, a mixture of propylene oxide and maraglas mixture 1:1 v/v, and maraglas mixture (Polysciences, Inc., Rydal, Pennsylvania). The maraglas mixture used for embedding consisted of ml proportions of maraglas 34, cardolite 10, dibutyl phthalate 5, and benzyl dimethylamine 1 (Freeman and Spurlock, 1962). A drop of fresh maraglas mixture was added to the bottom of a Beem plastic capsule, then nematode specimens were introduced and oriented, and maraglas mixture added to fill the capsule. The capsules then were incubated at 60 C for 48 hr. Blocks were trimmed and sections were cut to give bright gold to silver interference color with a glass knife in a Sorvall MT-2 Porter-Blum Ultra-Microtome. Sections were stained in 1% aqueous lead oxide for 5 min and then viewed under a HU-11A Hitachi electron microscope at 50 kv.

### RESULTS

Seven cuticular layers were seen, under the electron microscope, in cross and longitudinal sections of *T. martini* (Ibrahim, 1965). Nonswarming specimens of *T. martini* showed no morphological changes in the cuticular layers and the cuticle appeared intact (Fig. 1A). In many sections of swarming specimens morphological changes in the outer layers of the cuticle were detected. These changes probably started

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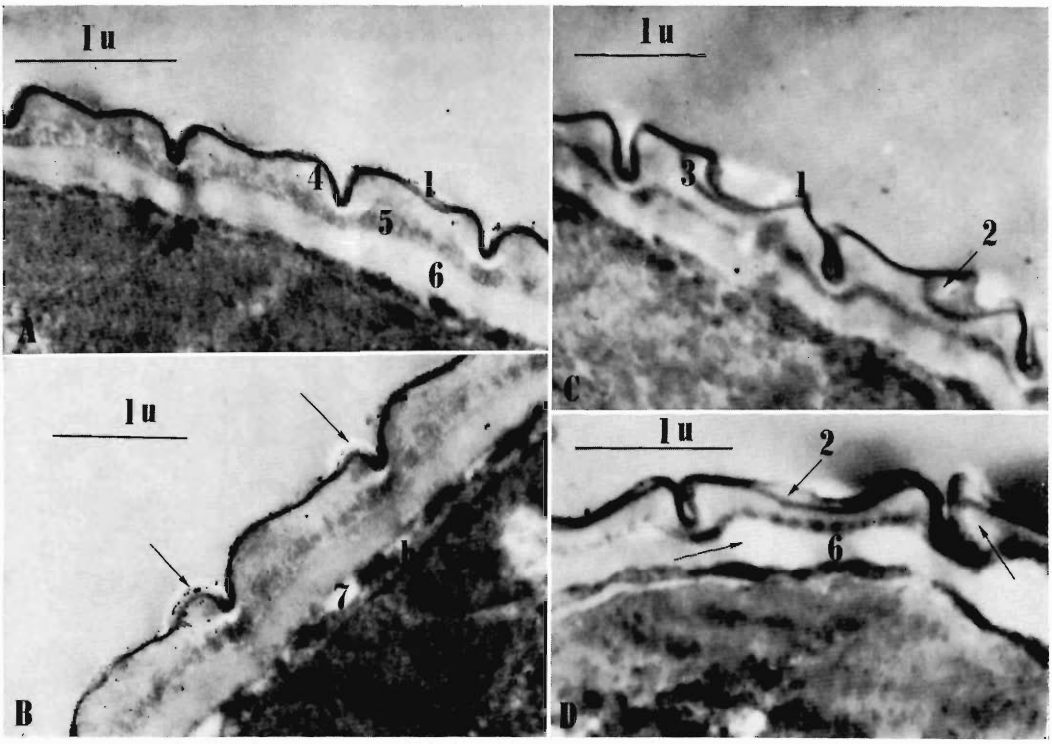


FIG. 1. *Tylenchorhynchus martini*, adult female. A. Longitudinal section through the cuticle of nonswarmer. B. Oblique longitudinal section through the cuticle of a swarmer, early state. Note the swelling of the outer layer at the edges of the interstrial regions (arrows). C. Oblique longitudinal section in the cuticle of swarmer, later state. D. Cross section of the cuticle of swarmer showing bright light-colored spots in the matrix and fiber layers (arrows), later state.

Abbreviations for figures: 1, external cortex; 2, internal cortex; 3, first boundary layer; 4, matrix; 5, second boundary layer; 6, fiber layer; 7, third boundary layer; h, hypodermis.

at the edges of the interstrial regions (Fig. 1B) as a swelling in the external cortex and separation of the two cortical layers from each other. In later stages these changes extended throughout the interstrial regions (Fig. 1C). In some specimens, bright, light-colored spots occurred in the matrix and fiber layers in addition to the changes in the cortical layers (Fig. 1D).

The morphological differences between swarming and nonswarming specimens of *T. martini* indicate that swarming may be initiated by an internal mechanism similar to that described for hatching and molting by Rogers (1960). Internal secretions may induce the morphological changes in the cuticle as they reach the outer layers. Special cuticular canals (Fig. 2) in the cuticle may be functioning as avenues for the internal secretions.

## SUMMARY

Morphological differences between the cuticle of swarming and nonswarming specimens of *Tylenchorhynchus martini* appear as morphological changes in some cuticular layers of swarming specimens. Such changes did not appear in the cuticle of nonswarming specimens. Morphological changes include swelling of the external cortex, separation of the two cortical layers, and bright, light-colored spots in the matrix and the fiber layers.

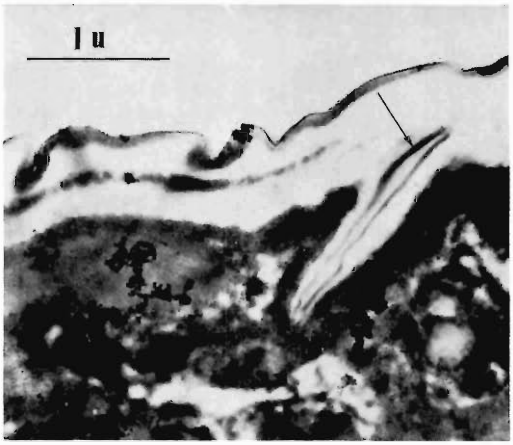


FIG. 2. *Tylenchorhynchus martini*. Oblique longitudinal section in the cuticle of swarming adult female showing a cuticular canal (arrow).


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## Notes on the Genus *Isolaimium* Cobb, 1920 (Nematoda), with Descriptions of New Species from South Africa

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The genus *Isolaimium* was described by Cobb in 1920, based on a single species *I. papillatum*, collected on Plummer's Island, Potomac River, Virginia, USA. This species has apparently never again been collected, and no other species were added until Timm described *I. stictochroum* in 1961. Shortly afterwards Andrássy (1962) gave a redescription of *I. papillatum*, based on a female from the banks of the Adige River in Italy.

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In our opinion the species described by Andrássy is not conspecific with *papillatum*, and is accordingly renamed *Isolaimium andrassyi* nom. nov., synonym *Isolaimium papillatum* of Andrássy 1962 (nec *Isolaimium papillatum* Cobb, 1920). *I. andrassyi* differs from *I. papillatum* in the much less well-developed transverse striae on the front part of the body. Although Cobb does not mention the striae in his description, his drawing clearly shows very prominent striae just behind the lip region. Calculated from Cobb's drawing, these striae

are approximately 1.2 $\mu$ apart, while Andrássy states that the very fine striae on his specimen are only 0.8 $\mu$ apart. Furthermore, Andrássy observed longitudinal lines on the posterior part of the body, which are apparently not present in *papillatum*. We do not consider it advisable to base a redescription of *papillatum* on specimens other than Cobb's types or else on topotypes, since the original description is so incomplete that it is virtually impossible to identify this species with certainty. Attempts to locate the type specimens among Cobb's slides at Beltsville have thus far been unsuccessful.

Recently three species of *Isolaimium* were collected in various localities in South Africa. It is interesting that while *papillatum* and *andrassyi* were from aquatic surroundings, *stictochroum* and the three South African species are from cultivated fields.

The taxonomic position of *Isolaimium* was discussed by Timm (1961) and Andrássy (1962). There is nothing we can add to this except to endorse Andrássy's view that this genus probably deserves to be put in a family of its own. The peculiar nature of the cephalic papillae has already been remarked on by Andrássy. Face views prepared of two of the South African species shed further light on the nature of these papillae. Normally one finds nerve fibers leading inwards from cephalic papillae, but in this case there appears to be thick-walled tubes leading backwards from the inner circle of six "papillae" (see Fig. 1B, C, E). In a face view a hollow lumen can be seen in these tubes. Two of these tubes come very close to the sensillae pouches, but no definite connection with the latter could be traced. Cobb could not locate the amphid apertures in his species, and neither could Andrássy find the amphid apertures on his specimen. In the three species which we studied the amphid apertures could also not be found. On the other hand, Timm observed small pore-like amphid apertures on *I. stictochroum*.

## DESCRIPTION OF NEW SPECIES

### *Isolaimium africanum* n. sp.
(Fig. 1A–H, Fig. 2B, C)

FEMALE ($n = 6$): L = 4.8 (3.9–5.7) mm; a = 70 (49–91); b = 18 (14–23); c = 81 (56–106); V = $^{11.5-14.7}$ 50 (48–53) $^{12.3-15.1}$.

MALE ($n = 6$): L = 4.7 (3.6–5.9) mm; a = 75 (58–93); b = 17 (14–21); c = 75 (59–90).

HOLOTYPE (female): L = 5.3 mm; a = 86; b = 23; c = 106; V = $^{11.5}$ 48 $^{12.3}$.

ALLOTYPE (male): L = 5.2 mm; a = 85; b = 18; c = 78.

Body lying in the shape of a letter (C) when relaxed. Body of uniform width over most of its length, tapering only towards the front part of the neck and the tail. Surface of cuticle marked by about 80 longitudinal striae, 1.3–1.8 $\mu$ apart around midbody, which occur over the entire length of the body except on the lip region. Transverse striae, also on the outer surface of the cuticle, occur from behind the lip region over the entire body. These striae are especially prominent just behind the lip region, where they are about 1.6–2 $\mu$ apart. Towards the base of the esophagus they became closer together and less prominent, and even more so over the rest of the body. On the front part of the neck the cuticle is divided into distinct blocks by the longitudinal and transverse striae. Head only slightly offset by a shallow depression. Lips not distinct rather closely amalgamated, the contour somewhat hexagonal in a face view. Lip region 21–27 $\mu$ wide, about half as wide as the body at the base of the esophagus. In the subcuticle just behind the lip region there occurs a darkish colored pod-shaped organ on either side of the neck. Stoma 95 to 134 $\mu$ long, cylindrical, with strongly cuticularized walls. In cross section the stoma consists of three cuticularized elements, forming a roughly triangular lumen. Stoma 3.6–4.9 times as long as width of lip region. Esophagus cylindroid, about one-third the corresponding body diameter, and 145–190 $\mu$ in length. Cardia elongate–conoid. Nerve ring situated at about 44% of length of esophagus, measured from base of stoma. Tail dorsally convex, ventrally concave, bluntly rounded, 48–69 $\mu$ long, 1.0–1.4 times the anal body diameter. Subcuticle thickened around terminus of tail, and longitudinal striae forming very distinct annules around the tail tip. No caudal papillae observed in the female, male with one postanal ventromedian papilla and a pair of subdorsal papillae. Rectum

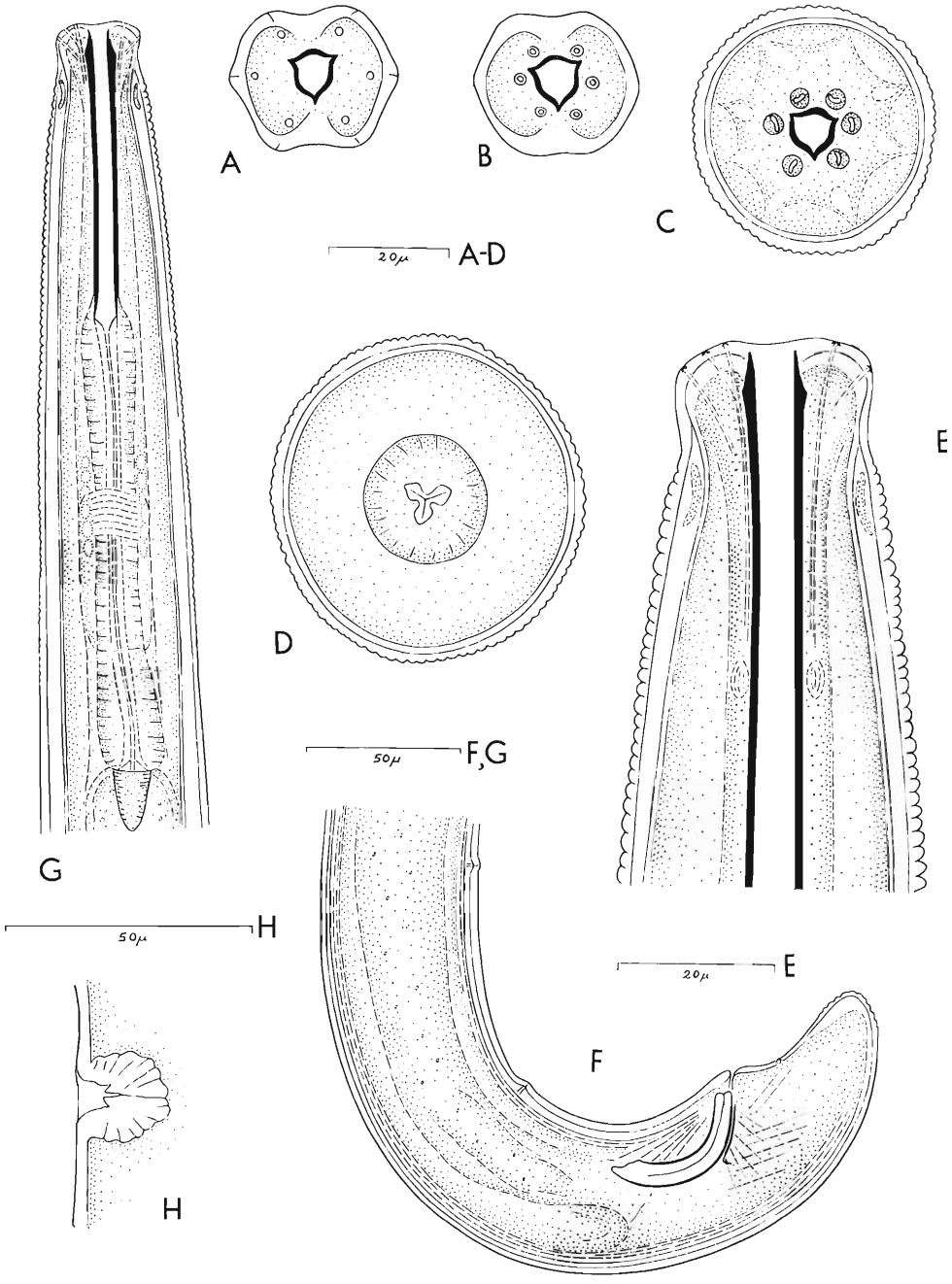


FIG. 1. *Isolaimium africanum* n. sp. A–D. Face view at level of cephalic papillae, slightly further backwards, middle of stoma, and esophagus, respectively. E. Head. F. Posterior part of male. G. Anterior part of body. H. Vulva and vagina.

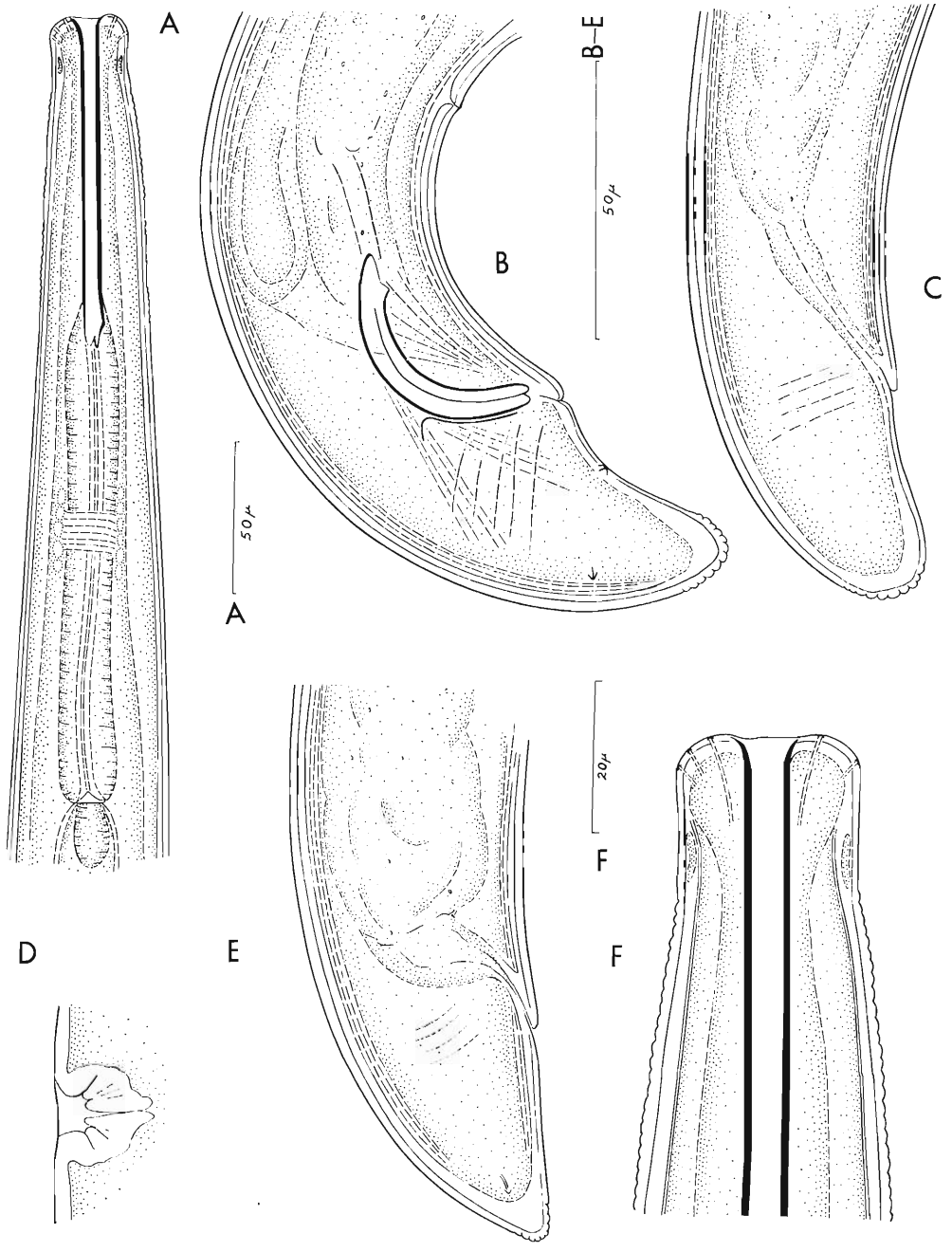


FIG. 2. A, D–F. *Isolaimium multistriatum* n. sp. A. Anterior part of body. D. Vulva and vagina. E. Female tail. F. Head. B, C. *Isolaimium africanum* n. sp. B. Male tail. C. Female tail.

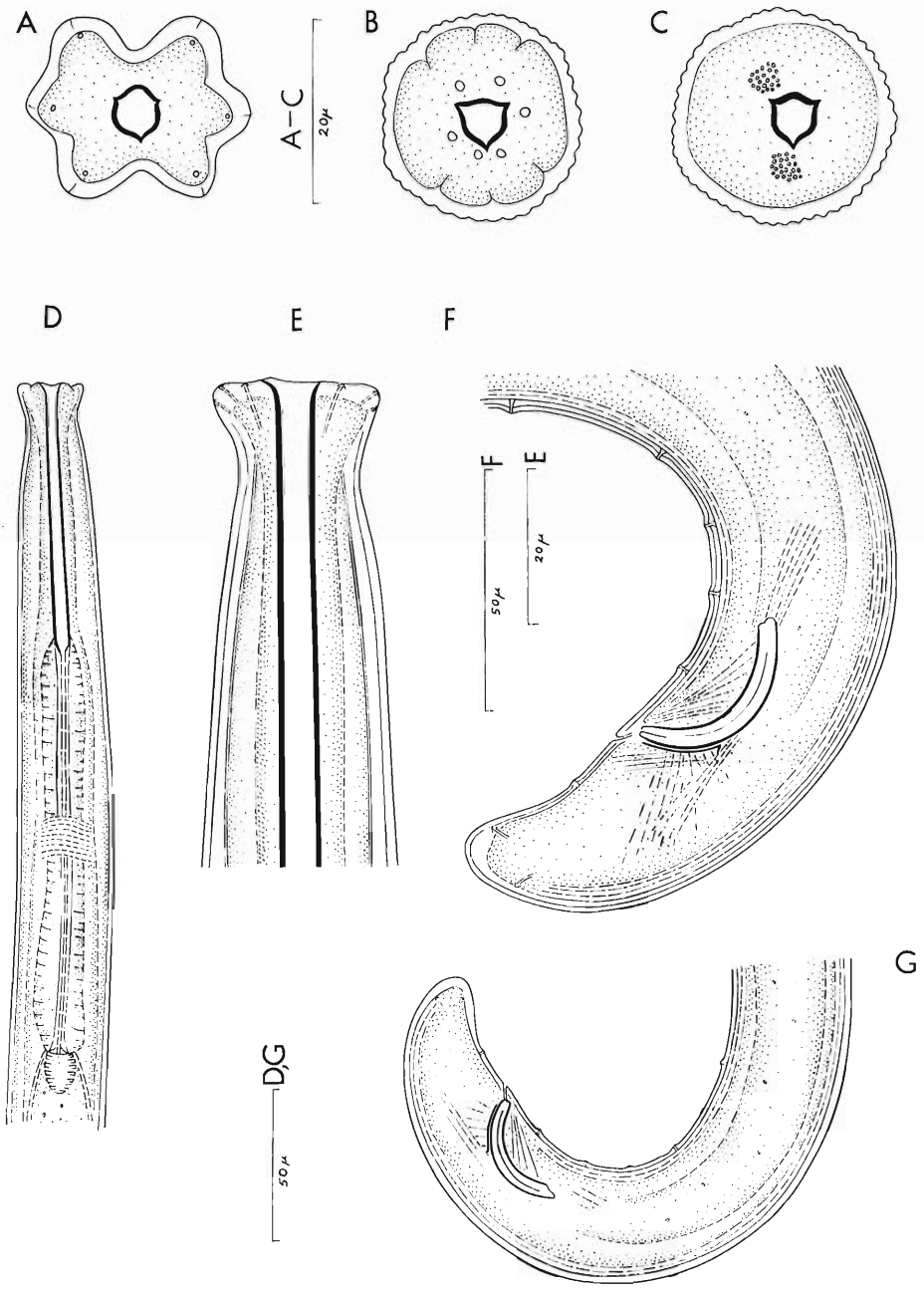


FIG. 3. *Isolaimium incus* n. sp. A–C. Face view at level of cephalic papillae, slightly farther backwards, and at middle of stoma, respectively. D. Anterior part of body. E. Head. F. Male tail. G. Posterior part of male.

slightly longer than anal body diameter. Prerectum not observed. No lateral field. Width of lateral cord equal to one-third the body diameter.

Female amphidelphic. Vulva large, circular, sunken below the surface, with small inner lips; vagina short, broad; gonads dorylaimoid in appearance, with large undifferentiated uterus, separated from oviduct by sphincter muscle; ovary reflexed. Uterine egg measures 65–70 by 38–42 $\mu$, with egg shell 3 $\mu$ thick.

Spicules strongly arcuate, measuring 52–61 $\mu$ along the curved median line. No lateral guiding pieces. Gubernaculum thin, linear, more than one-third as long as spicules, and provided with a prominent backward-directed process at its proximal end. No adanal supplements, but a ventromedian series of two to six, irregularly spaced.

TYPE LOCALITY AND HABITAT: The type specimens were collected during September 1963 and again in June 1964 on various localities on the farm Elandsfontein of the South African Forest Investments Co., near Sabie in the Transvaal, and were found in soil in *Pinus patula* and *Eucalyptus saligna* plantations.

TYPE SPECIMENS: Holotype on slide 4667, allotype on slide 2897, paratypes on slides 2896, 2913–2916, 4666, 4668, and 4670. One female paratype deposited in the collection of the USDA, Nematology Investigations, Beltsville, Maryland.

DIAGNOSIS: *I. africanum* n. sp. differs from *I. papillatum* Cobb and *I. andrassyi* nom. nov. in the presence of longitudinal striae on the surface of the cuticle. From *I. andrassyi* it further differs in the presence of prominent transverse striae on the front part of the neck, and in the shape of the tail. From *I. stictochroum* it differs in the shape of the lip region which is confluent in *stictochroum* but slightly offset in *africanum*, and in the presence of prominent transverse striae on the surface of the cuticle. From *multistriatum* n. sp. it differs in the smaller number of longitudinal striae, differently shaped tail, and slightly offset lip region.

*Isolaimium multistriatum* n. sp.
(Fig. 2A, D–F)

FEMALE ($n = 4$): L = 4.8 (4.1–5.2) mm; a = 71–74; b = 18 (14–19); c = 84 (61–93); V = 50 (49–53).

HOLOTYPE (female): L = 5.0 mm; a = 74; b = 19; c = 92; $V = {}^{14.5}53{}^{15.8}$.

Description similar to that of *Isolaimium africanum* except for the following differences: Lip region not offset, but shaped as in *I. papillatum*, with the transverse striae beginning somewhat further backwards than in *africanum*. Transverse striae much less distinct. Longitudinal striae at least 120 in number and about 1 $\mu$ apart. Stoma 100–106 $\mu$ long. Tail convex–conoid, more pointed than in *africanum*. Caudal papillae observed near the tail terminus. Several eggs occur together in the uterus, eggs measuring 78–84 by 42 $\mu$. Male not known.

TYPE LOCALITY AND HABITAT: Four females and several juveniles were collected in a maize field on the farm "Rustig" of Mr. C. Kriel in the Clocolan district of the Orange Free State, December 1963.

TYPE SPECIMENS: Holotype on slide 4010, paratypes on slide 4017.

DIAGNOSIS: *I. multistriatum* n. sp. differs from its nearest relative *I. africanum* n. sp. in the characters mentioned above.

*Isolaimium incus* n. sp.
(Fig. 3A–G)

MALE ($n = 3$): L = 3.0–3.7 mm; a = 79–87; b = 16; c = 71–79.

HOLOTYPE (male): L = 3.7 mm; a = 87; b = 16; c = 79.

Body ventrally curved, especially in posterior part; of uniform width over most of its length, tapering only towards front part of neck and tail. Surface of cuticle with about 50 longitudinal striae, 1.3–1.5 $\mu$ apart around midbody, occurring over the entire length of the body except on the lip region. Surface of cuticle also marked by transverse striae, but these are very indistinct, even on the front part of the neck. Lip region truncate, expanded, set off by broad depression. Lips rather well separated, hexagonal in a face view. Lip region 22 $\mu$ wide, which is two-thirds the width of the body at the base of the esophagus. Stoma 84 $\mu$ long. Esophagus

cylindroid and about 135 $\mu$ long. Cardia heart-shaped, about 12 $\mu$ long. Nerve ring situated at 47% of the length of the esophagus. Tail conoid, dorsally convex, ventrally concave, the terminus bluntly rounded. Tail 37–47 $\mu$ long, 1.2–1.4 times the anal body diameter. Subcuticle slightly thickened around terminus of tail. Tail with a postanal ventromedian papilla and two subterminal pairs of papillae. No lateral field. Lateral cord about one-fourth as wide as the body.

Spicules strongly arcuate, measuring 44–47 $\mu$ along the curved median line. No lateral guiding pieces. Gubernaculum more than one-third as long as spicules, thin, linear, and provided with a small backward-directed process at its proximal end. No adanal supplements, but a ventromedian series of three to five, the first of which occurs opposite the middle of the spicules.

Female not known.

TYPE LOCALITY AND HABITAT: Collected in a maize field in the Bothaville district in the Orange Free State, December 1964.

TYPE SPECIMENS: Holotype on slide 5535, paratypes on slides 5536 and 5682.

DIAGNOSIS: *Isolaimium incus* n. sp. can be distinguished by its expanded lip region, which is different from any other species in the genus.

KEY TO SPECIES OF *Isolaimium*

1. Longitudinal striae absent, or present only on posterior part of body ..... 2
   Longitudinal striae over entire body .... 3
2. Transverse striae conspicuous behind lip region ..... *papillatum*
   Transverse striae very fine ..... *andrassyi*
3. Lip region expanded and set off by a pronounced depression ..... *incus*
   Lip region not expanded, confluent, or set off by a slight depression only .... 4
4. Fine transverse striae in subcuticle .... ..... *stictochroum*
   Conspicuous transverse striae on surface of cuticle ..... 5
5. Longitudinal striae more than 120; tail conoid ..... *multistriatum*
   Longitudinal striae about 80; tail bluntly rounded ..... *africanum*

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## Experimental Transmission of *Histomonas meleagridis* and *Heterakis gallinarum* by the Sow-bug, *Porcellio scaber*, and Its Implications for Further Research

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The epizootiology of poultry blackhead has not been completed, especially as it concerns the modes of natural transmission of the causal agent, the protozoon, *Histomonas meleagridis*.

Studies by Graybill and Smith (1920), Smith and Graybill (1920), and others established that *H. meleagridis* (histomonads) can be transmitted from bird to bird by the embryonated eggs or larvae of a parasitic invertebrate, the poultry cecal nematode, *Heterakis gallinarum* (heterakids). Other investigations disclosed that heterakids and histomonads, and therefore blackhead, can be disseminated by certain free-living invertebrates. For example,

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infections of one or the other or both parasites, with or without signs of blackhead, were observed in birds after they were fed (a) earthworms and houseflies from poultry or pheasant yards (Curtice, 1907; Ackert, 1917; DeVolt and Davis, 1936; Madsen, 1962, Lund, Wehr, and Ellis, 1963), and (b) houseflies, fleshflies, and grasshoppers that had been kept in contact with embryonated eggs of heterakids obtained from sources where blackhead was present (Frank, 1953).

This information makes suspect as a potential disseminator of these parasites any free-living invertebrate that may be eaten by poultry. One such invertebrate is *Porcellio scaber*, the sow-bug (bugs), a crustacean that feeds on decaying or putrefying organic matter, and is commonly found in poultry yards. Such bugs might serve as sources of infection for birds that swallow them. Since this possibility has not been tested, a study was made to determine if *P. scaber* could transmit heterakids and histomonads to turkeys under experimental conditions.

## MATERIALS AND METHODS

TURKEYS: Beltsville Small White poults, 6 weeks old, were used. From hatching to necropsy they were maintained in wire-bottom cages, under conditions designed to preclude extraneous infections of histomonads and heterakids. Clean feed and water were provided throughout.

SOW-BUGS: Two lots (I and II) of adult sow-bugs were collected, each from a single "wild" colony. Each lot of bugs was maintained in a separate terrarium, a 500-ml beaker. The soil was overlaid with several small pieces of partially decayed wood. To maintain moisture, the underside of the wood and the soil were sprayed lightly with water as needed. Scraps of putrefying raw beef for food were placed each day beneath wood where the bugs congregated.

TESTS FOR NATURAL INFECTIONS OF PARASITES: Thirty-four bugs of Lot I were force-fed, alive, to 17 poults, 2 per bird; 50 of Lot II were force-fed, alive, to 10 poults, 5 per bird. Sixteen days later, necropsies were performed on the birds.

NECROPSIES: Livers and ceca were inspected for signs of blackhead. For each bird, two smears in physiologic saline were prepared from the contents of each cecum and examined for histomonads and other protozoa by phase-contrast microscopy. The remainder of the contents and the scraped-off mucosa were suspended together in warm saline, sedimented, and examined for heterakids; appropriate examinations were made for other helminths.

EXPOSURE OF SOW-BUGS TO PARASITES: Each day for 2 weeks, 1 ml of a rich suspension of heterakid eggs in water was distributed on the meat, on the underside of the wood, and on the soil of each terrarium. These eggs were from pooled cultures that were being used successfully to transmit heterakids and histomonads to poults and which had produced clinical blackhead in them under experimental conditions.

TESTS OF TRANSMISSION: After exposure, 16 bugs of Lot I and 145 of Lot II were washed in wire baskets for 5 min under a strong stream of warm water from a faucet, then in five changes of water in beakers to dislodge eggs that might be adhering to the bugs. Those of Lot I were then force-fed, alive, to 8 poults, 2 per bird; those of Lot II were force-fed, alive, to 29 poults, 5 per bird. Fifteen other poults not fed bugs were kept as controls. The test and control birds were observed for signs of blackhead for 14 days and were then necropsied.

MICROSCOPIC EXAMINATION OF SOW-BUGS AND TESTS ON TERRARIA: After the bugs exposed to parasites were fed to poults, five remained in each terrarium. They were transferred to clean terraria for 7 days. They were then dissected and examined microscopically for eggs and larvae of heterakids and for histomonads to indicate the mode of transmission.

The surface soil and scrapings from the underside of the wood of each original terrarium were mixed in feed and administered to a poult that was later examined post-mortem. This examination indicated whether the parasites were viable during the time the bugs were associated with them.

## RESULTS

All the birds fed sow-bugs and the ones not fed these crustaceans remained healthy and signs of blackhead were not detected. All transmissions that were obtained were of histo-

monads and heterakids together. The amount of transmission was small, about the same for the two lots of bugs. For that reason the findings are treated as a unit; the pertinent ones are listed below.

*Birds fed sow-bugs exposed to heterakids and histomonads*

| | |
|---|---|
| Number of birds | 37 |
| Number parasite-free at necropsy | 29 |
| Number with blackhead signs | 0 |
| Number with heterakids and histomonads at necropsy | 8 |
| Histomonads in cecal smears | 1/8–10 fields |
| Heterakids per bird | 1–3 |
| Birds with heterakids of both sexes | 4 |

*Birds fed sow-bugs not exposed to heterakids and histomonads*

| | |
|---|---|
| Number of birds | 27 |
| Number with parasites | 0 |
| Number with blackhead signs | 0 |

*Controls (birds not fed sow-bugs)*

| | |
|---|---|
| Number of birds | 15 |
| Number with parasites | 0 |
| Number with blackhead signs | 0 |

No helminth parasites other than heterakids were found; protozoa other than histomonads, if present, did not occur in numbers sufficient to be revealed by the method of examination employed.

The two birds fed materials from the original terraria developed blackhead and died. At necropsy, signs of blackhead were observed in each, and the cecal contents contained massive numbers of histomonads; 193 heterakids were recovered from one of the birds, 247 from the other.

Neither nematode eggs or larvae nor histomonads were found by dissection and microscopic examination of the five bugs of each collection that were kept in clean terraria for 7 days after exposure to eggs of heterakids.

## DISCUSSION

Under conditions of this study, the sow-bug, *Porcellio scaber*, did not prove to be a good transmitter of the nematode, *Heterakis gallinarum*, and the protozoon, *Histomonas meleagridis*, and therefore blackhead. Only eight of 37 poults fed bugs exposed to these parasites became infected. The infections were small, and none of the birds developed signs of blackhead. The bugs had been given a good opportunity to ingest sizable numbers of heterakid eggs, some of which must have contained histomonads. This is indicated by the fact that poults fed materials from the terraria used to expose the bugs developed blackhead and died, and large infections of heterakids and histomonads were found in them.

The failure to find heterakids and histomonads in poults fed sow-bugs not exposed to these parasites indicates that natural infections of them did not exist in the colonies from which the bugs were collected.

Such transmission as was obtained probably was from heterakid eggs in the gut of the bugs at the time they were fed to poults, and was therefore mechanical; there was no indication that the bugs served as a host for the heterakids and histomonads. Neither nematode eggs nor larvae nor histomonads were found in the 10 exposed bugs that were dissected after they had been kept for 7 days in clean terraria. Had the eggs hatched in the gut of the bugs and the larvae migrated to the surrounding tissues, they should have persisted there and some should have been found. *P. scaber* is capable of serving as an intermediate host as was shown by Cram (1930) in the case of *Dispharynx spiralis*, a nematode parasite of birds.

In these attempts at transmission, there were factors that may have influenced the results. Small, presumably young, bugs became numerous in the terraria, but in spite of this increase the number of bugs in each terrarium decreased with time. When transmission feedings were made, it was necessary to use some smaller bugs as well as some larger ones. It is not known if the smaller bugs swallowed eggs in any numbers. Another potential factor is the proportion of the heterakid eggs that may be expected to have contained histomonads, about one in 200 according to Lund and Burtner (1957). This may have influenced transmission, especially if small numbers of eggs were contained in the gut of individual bugs at any one time.

Although the amount of transmission that was obtained was too small to appear impor-

tant, there may be a long-range significance. Of the eight birds that became infected, four harbored heterakids of both sexes. If these worms had been permitted to develop to sexual maturity, fertile eggs, some with histomonads, might have been produced. Therefore, from a small beginning, such as produced by one or a few sow-bugs, the infections might, in time, build up to serious proportions under flock conditions.

There are other potential avenues of transmission of heterakids and histomonads, and therefore blackhead, that may offer implications for further research. These include those free-living invertebrates and those ectoparasites that at some stage of their development may be coprophagous. Some histomonads in heterakids are said to occur in the reproductive organs, and may have an affinity for reproductive tissues. If that is the case, the protozoon may have an affinity for the reproductive tissues of other gastrointestinal helminths as well. Until all forms capable of carrying histomonads and heterakids are identified, and their potential for transmission evaluated, it may be impossible to develop measures of maximum efficiency for the control and elimination of blackhead.

## SUMMARY

Sow-bugs, *Porcellio scaber*, were tested experimentally for their ability to transmit the protozoon, *Histomonas meleagridis*, and its vector, the parasitic nematode, *Heterakis gallinarum*. Sow-bugs from natural colonies were housed in terraria, exposed to histomonad-bearing eggs of heterakids, and fed to susceptible poults. None of the 37 birds developed signs of blackhead; at necropsy, 8 harbored light infections of heterakids (1 to 3 per bird) and histomonads (1 per 8 to 10 microscope fields in cecal smears). Two other poults fed materials from the experimental terraria died of blackhead, and harbored massive infections of histomonads and heterakids, showing that the parasites used were viable. Twenty-seven birds fed bugs not exposed to parasites, and 15 others not fed bugs (the controls) remained healthy and free of parasites. Neither nematode eggs nor larvae nor histomonads were found in 10 exposed bugs that were kept in clean terraria for 7 days, then dissected for microscopic examination. Apparently, the transmission obtained was mechanical, from unhatched heterakid eggs in the gut of the bugs at the time they were fed to poults. Some implications for further research on transmission are discussed.

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# Morphological Variant of *Tetylenchus joctus* Thorne (Nemata: Tylenchida) Associated with Cultivated Blueberries in Indiana*

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*Tetylenchus joctus* Thorne, 1949, has been reported to be frequently associated with the roots of cultivated blueberries (*Vaccinium corymbosum* L.) in New Jersey (Hutchinson *et al.*, 1960; Hutchinson *et al.*, 1961), and with cranberries (*V. macrocarpon* Aiton) in Massachusetts (Zuckerman 1960, 1961). Hutchinson and his co-workers (1961) reported finding very high populations (up to 6,000/pint of soil) in one planting of blueberries near New Lisbon, New Jersey, where the population remained abundant over a 4-year period. Zuckerman (1961) has shown that *T. joctus* feeds on the epidermal cells of the roots of cranberry plants, causing a collapse of the cells. Though the cultivation of blueberries constitutes an important industry in Michigan, and a growing industry in Indiana, this species has not previously been reported to be associated with the crop in either of these states. Fairly extensive sampling of soil in blueberry plantings has been carried out in Michigan (Knierim, 1960).

## MATERIALS AND METHODS

Soil samples were taken in July and August 1965 from blueberry plantings in two locations in northern Indiana. On one farm in North Judson, Indiana, the following soil samples were taken, by means of a sampling probe, from around the base of plants in different areas of the farm: (1) dark sandy soil, blueberry variety Jersey, planted about 10 years previously; (2) dark sandy soil, blueberry variety Burlington, planted about 17 years previously; (3) black sandy soil containing more organic matter (muck) than the first two samples, blueberry variety Jersey; and, (4) uncultivated dark sandy soil, wild blueberries. At the other location (La Paz, Indiana) two soil samples were taken, both a highly organic peat soil, and both from around plants of the variety Jersey.

One-pint subsamples of each soil sample were processed using a combination of sieves and Baermann funnels. Aliquots of the nematodes recovered were counted, and the counts averaged. Nematodes which were to be measured were relaxed by gentle heat, killed, and fixed in formalin–acetic–alcohol (FAA), dehydrated to pure glycerine, and mounted in glycerine using Thorne's (1961) methods.

## RESULTS

Very few plant parasitic species were recovered from the soil samples, with the exception of the sample from North Judson which was taken from the 10-year-old planting of the variety Jersey in the dark sandy soil. In this soil sample there were present an average of 7,000 specimens of a *Tetylenchus* species per pint of soil. No specimens of *Tetylenchus* were recovered from any of the other soil samples.

## IDENTITY OF THE *Tetylenchus* SPECIES

Examination of the specimens showed that this was a species similar to *T. joctus*; however, certain details of the tail in both males and females did not follow the description. *T. joctus* was described as having the terminus subacute, and with no striations on the terminus (Thorne, 1949). The shape of the tail was given as one of the diagnostic characters of the species. The specimens from Indiana, however, have a terminus that is usually acute, sometimes almost mucronate, as was described for *T. productus* Thorne, 1949. In addition, in the Indiana specimens the termini are striated and are similar in appearance to those described for *T. annulatus* Merny, 1964. The tail shape is similar for both males and females (Fig. 1).

In other characteristics the Indiana specimens follow closely the description for *T.*

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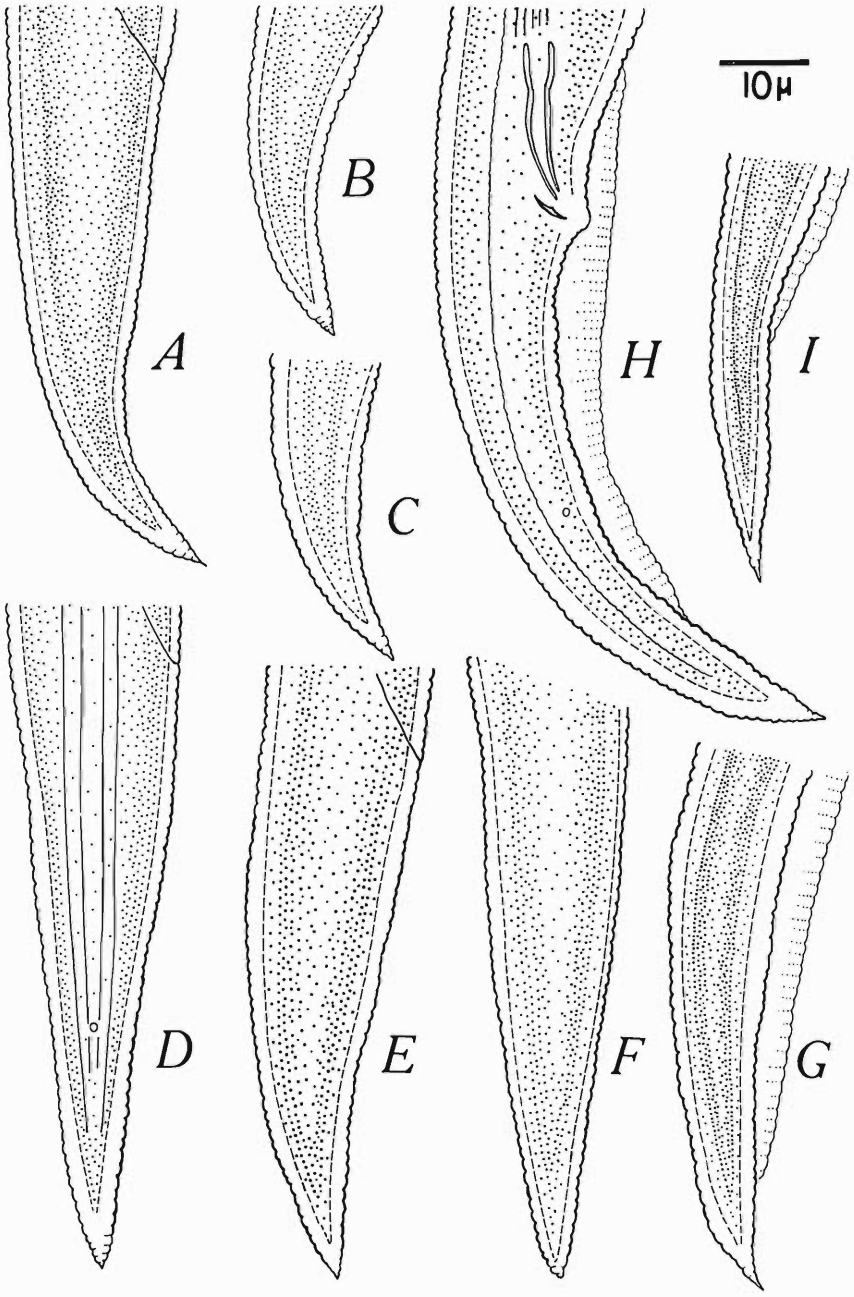


FIG. 1. Variations in tail observed in Indiana specimens of *T. joctus*. A–F, female tails; G–I, male tails.

*joctus*, a species originally described from specimens collected in Wrangell, Alaska. A comparison of the standard measurements and ratios is given:

*Tetylenchus joctus* Thorne, 1949

FEMALE: 0.7 mm; a = 30; b = 4.5; c = 9.4; V = $^{31}55^{38}$; stylet: 15 $\mu$.

MALE: 0.6 mm; a = 30; b = 4.5; c = 9.0; T = 70.

*Tetylenchus joctus*, Indiana Specimens

FEMALE ($n = 10$): 0.67 mm (0.61–0.73); a = 31 (28–34); b = 5.4 (4.6–6.1); c = 12 (10–18); V = $^{26}56^{24}$ ($^{23\text{-}32}55\text{–}59^{21\text{-}27}$); stylet = 15 $\mu$ (15–16).

MALE ($n = 10$): 0.60 mm (0.56–0.64); a = 31 (28–39); b = 4.8 (4.5–5.3); c = 10 (9–12); T = 45 (43–50); stylet = 15 $\mu$ (15–16).

Because of the similarity of the Indiana specimens to *T. joctus*, it has been decided to consider this geographically disjunct population as a morphological variant of that species and to record herewith the variations described above.

## DISCUSSION

The fact that *T. joctus* has not previously been reported in the north-central part of the U.S., though it appears to be a frequently encountered species in blueberry and cranberry plantings in the East (Mai *et al.*, 1960), leads us to suspect that the species was imported to Indiana on blueberry plants. The Indiana grower who owned the planting in which the species was found reported that the particular area in question had been planted before he acquired the property, but that he was certain that all of the original seedlings had come from either Michigan or New Jersey. Our hypothesis tends to be confirmed by the fact that we have not found the species around wild blueberries, even in close proximity to the infested cultivated area. *T. joctus* has been found in association with wild blueberries in New Jersey (Hutchinson *et al.*, 1961).

There is no reference in any of the published records of the occurrence of this species in New Jersey or Massachusetts to the atypical tail features described above. However, we have ascertained by correspondence with Professor Gerald Thorne, who has examined specimens from a New Jersey population, and from Indiana, that all of these specimens from both localities exhibit the atypical tail characteristics which we have described here on the basis of Indiana materials. This strengthens the belief that the species has been introduced to Indiana.

## SUMMARY

Although *Tetylenchus joctus* Thorne, 1949, has been reported to be a frequent associate of blueberries and cranberries in the eastern United States, it has not previously been discovered in blueberry plantings in the north central region. Specimens recovered in large numbers from soil around blueberry plants in one location in Indiana differed in certain characteristics from *T. joctus* as originally described. These differences are detailed and the population recorded as a morphological variant of *T. joctus*.

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# Notes on the Life History of *Pleurogonius malaclemys* Hunter, 1961 (Trematoda: Pronocephalidae) from Beaufort, North Carolina, with a Description of the Cercaria

WANDA SANBORN HUNTER*

## SUMMARY

A monostome cercaria developing in the marine snail, *Nassarius obsoleta* (Say), in the Beaufort, North Carolina area, is proved to be the larva of *Pleurogonius malaclemys* Hunter, 1961. This is the first marine cercaria definitely determined to be the larva of an adult of the Family Pronocephalidae Looss, 1902. Morphology of the metacercariae which commonly are encysted on and under the opercula of the snail host, as well as results of feeding experiments with the definitive host, *Malaclemys terrapin centrata* (Latreille), 1802, validate the above statement.

## MATERIALS AND METHODS

Active, freshly emerged cercariae were killed in boiling seawater and measured under cover glass with the minimum amount of water necessary to prevent distortion from pressure; this method is that used by Cable (1956). Mature living cercariae could not be studied successfully under cover slip pressure due to their habit of rapid encystment when in contact with any suitable substrate. To facilitate observation of internal organs, cercariae were allowed to encyst on a slide and dissected out of their cysts within 2 or 3 min. This method allowed most of the cystogenous cells to empty, but did not alleviate entirely the difficulties of observation due to body pigments. Development of the excretory system was studied in living worms obtained by crushing the snail hosts; young, developing cercariae were dissected out of the rediae. Sections of mature as well as developing cercariae were made; hematoxylin and eosin staining was used.

Metacercariae of different ages were studied after removal from their cysts. These were photographed alive and then preserved in hot AFA solution under minimum cover glass pressure. Adult worms from experimentally fed turtles also were photographed before killing in hot AFA. Measurements of both the metacercariae and adults were made on stained and mounted specimens. Whole mounts were stained with either Semichon's or Ballard's. Photomicrographs of all living stages were made with a Polaroid camera; this method is particularly good for recording details of the excretory system which constantly vary in visibility.

## DESCRIPTION OF CERCARIA

Measurements in mm from 10 heat-killed specimens:

| | *Range* | *Average* |
|---|---|---|
| Body length | 0.493–0.554 | 0.525 |
| Body width (immediately posterior to collar) | 0.208–0.281 | 0.227 |
| Oral sucker | | |
| Longitudinal diameter | 0.040–0.054 | 0.049 |
| Transverse diameter | 0.038–0.054 | 0.045 |
| Tail length | 0.478–0.622 | 0.572 |
| Tail width (near base) | 0.053–0.068 | 0.059 |

Large, trioculate, monostome with smooth cuticle. Cephalic collar inconspicuous with ventral lobes approximately one-fifth to one-fourth of body length; lobes widely separated posteriorly, but joined medially behind oral sucker. Heavily pigmented body tends to be concave ventrally. Anterior end bluntly tapered with terminal, weak, subspherical oral sucker; body widens abruptly posterior to eyespots. Sides straight from posterior collar margins to rounded posterior end. Inconspicuous cup-shaped protrusible, apparently glandular, locomotor structures at posterolateral borders of body, extending beyond level of tail insertion. Cystogenous glands, filled with granular opaque material, immediately be-

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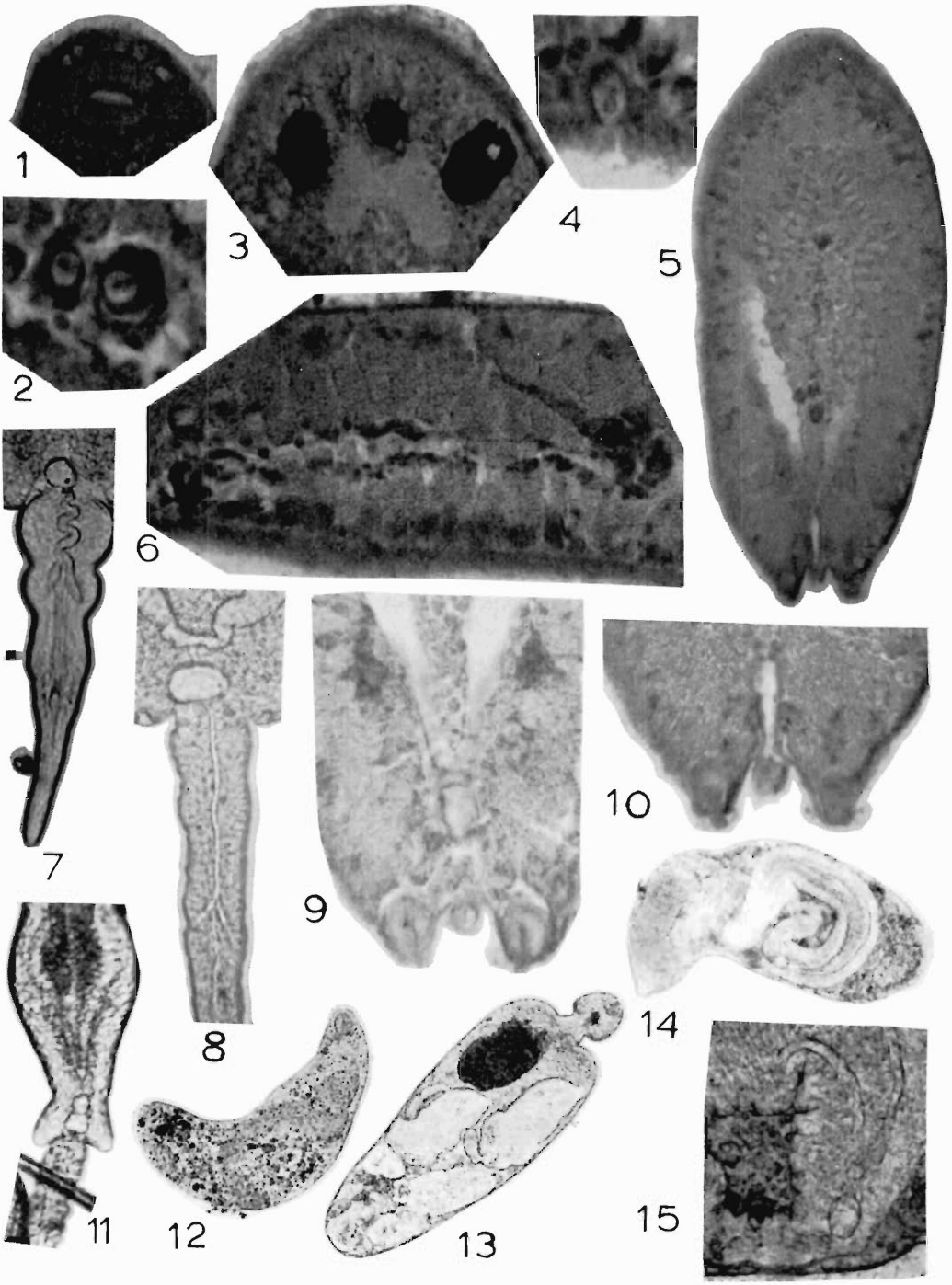

neath entire body surface except for extreme anterior end. Six cephalic glands surround oral sucker and their ducts open at extreme anterior end (Fig. 1, 3, 29). Median eyespot smaller, more irregular, less organized, usually lacks lens, and lies slightly anterior to other two, close to oral sucker. The three eyespots closely associated with bilobed ganglionic mass of nervous system (Fig. 3, 18, 29).

Pharynx lacking, esophagus thin, long, bifurcating posterior to collar in second one-fourth of body length. Cecae long, with irregular thick-walled diverticula throughout their length; diverticula more prominent and regular in anterior parts of cecae (Fig. 5, 17, 26).

Primordia of all genital organs present; two small extratesticular masses widely separated from each other in posterior part of body (Fig. 9, 26, 27); primordia of vitellaria anterior and lateral to testes (Fig. 26, 27). A more conspicuous median line of cells (Fig. 5, 6, 17, 18, 26) extends anteriorly from position of future adult ovary–ootype complex (Fig. 5, 6, 26, 27); this begins in front of excretory bladder and runs into anterior half of body where it turns to left and ends in post-bifurcal region of future genital pores. Rudiments of male and female ducts (Fig. 2), including one interpreted as Laurer's canal (Fig. 6, 26, 27) (not observed in adult, Hunter, 1961), as well as location of future genital pores, can easily be traced in sections (Fig. 4, 26, 27).

Most prominent part of excretory system consists of two lateral collecting tubes, filled with refractile bodies. In collar region, these ducts unite by a very small tubule to complete cycloid system characteristic of Family (Fig. 26, 28). Posteriorly large ducts meet and connect with bladder by thin-walled duct which regularly expands to form an anterior bladder, or vestibule; this alternates in pulsations with thicker-walled posterior bladder. (See Fig. 7, 8, 11, 14, 15, 16.) Therefore, bladder variable in shape, definitely of two parts, and when empty disappears from view and arms of large collecting ducts may then be easily mistaken for parts of a Y-shaped bladder. In a mature cercaria, the heavy-lipped excretory pore is in the dorsal wall of the bladder, some distance from the posterior margin of the body (Fig. 26); it is nonfunctional until the tail is lost. Small caudal atrium present; its two posterolateral openings function as excretory pores until tail is cast off at encystment (Fig. 10, 11, 26). Caudal excretory tubes prominent in developing forms (Fig. 7, 8), but at emergence, no evidence of a caudal duct or pores. Branches of main collecting ducts as described for adult (Hunter, 1961) visible in living forms. Flame cell pattern expressed as $2(3 + 3 + 3) + (3 + 3 + 3)$ (Fig. 28).

Tail straight, slightly longer than body, tapering gradually to tip. Few, irregularly scattered, small cells throughout length. Few, long, and somewhat spiral strands run lengthwise, independent of scattered cells. Cuticle with fine striations.

Development occurs in simple rediae (Fig. 12, 13, 14) which contain much yellow–orange and brown pigment, and which have a relatively long, wide gut. Young rediae often show marked constriction near anterior end. Birth pore inconspicuous, relatively close to pharynx. Cercariae leave rediae at early stage and mature in tissue spaces of host. Usually but two or three (often only one) cercariae were found developing in each redia; several small germ balls present in rediae with advanced larvae. No second-generation rediae found.

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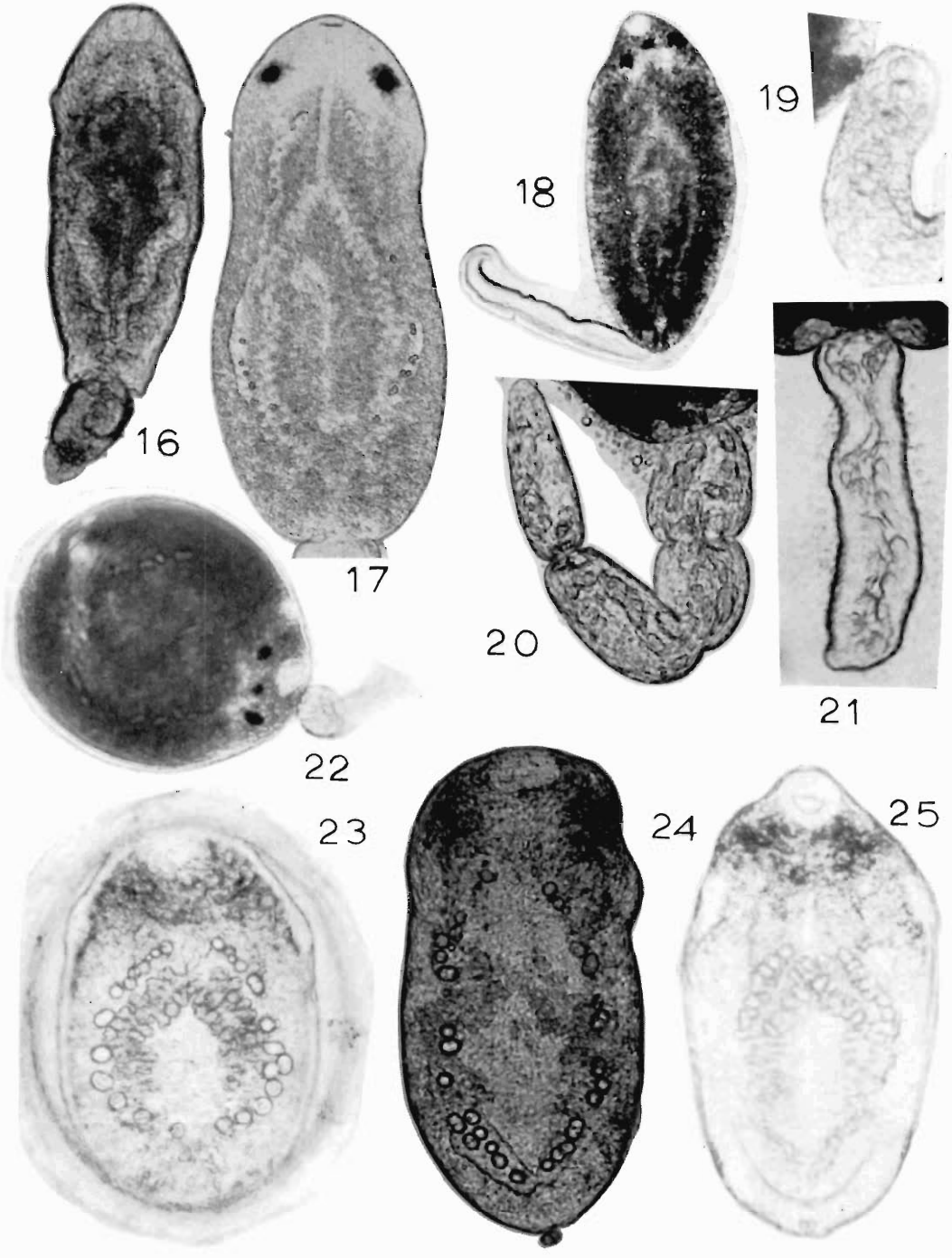
FIGS. 1–15. 1. (Frontal sec.) Ducts of cephalic glands. (200×). 2. (Cross sec.) Male and female ducts immediately posterior to developing cirrus pouch. (970×). 3. (Frontal sec.) Cephalic glands, ganglia, eyespots. (430×). 4. (Sagittal sec.) Cirrus pouch and genital pore area. (970×). 5. (Frontal sec.) Gut, genitalia, bladder. (200×). 6. (Sagittal sec.) Genital ducts, ootype region, Laurer's canal. (430×). 7. Live, developing cercaria ex snail. Thick-walled part of excretory bladder and caudal ducts. (150×). 8. Live, developing cercaria. Bladder and caudal ducts. Note contracted anterior bladder. (200×). 9. (Frontal sec.) Bladder, ducts, testes, locomotor organs. (430×). 10. (Frontal sec.) Bladder, atrium, and atrial pores. (430×). 11. Live cercaria ex redia. Two parts of bladder and atrial pores. (100×). 12. Live redia. (48×). 13. Live redia with young cercariae. Note constriction. (48×). 14. Live redia with older developing cercariae. Note double excretory bladder. (48×). 15. Live cercaria within redia. Note thicker wall of posterior part of bladder. (150×).



## DISCUSSION OF CERCARIA

Infected snails with emerging cercariae are to be found throughout the year, being somewhat more prevalent during the late fall and early months. The percentage of infected snails with emerging cercariae when isolated in the laboratory is usually less than 1%, although two collections (in November and December of different years) from isolated pens where infected turtles had been confined showed unusually high 3.3 and 4% infections. In any area where infected snails are found, cysts are conspicuous on and under opercula of both infected and noninfected snails.

In the laboratory, midday is the optimum time for emergence of cercariae; relatively few emerge daily as would be expected from the observations of the numbers developing within each redia. The cercariae are comparatively slow, often steady swimmers, moving in circles by means of violent tail-lashings. When they stop swimming, they soon encyst on snails as well as on the bottom of the glass culture dishes in which the hosts have been isolated. In the laboratory, many of the cercariae fail to encyst and die within a few hours. Failure of encystment of many cercariae suggests the premature emergence of some of the larvae due to laboratory conditions. Bioculate and trioculate cercariae are found at the same time and from the same snail host, the trioculate being the more numerous. During development, the poorly organized third eyespot appears after the more posterior two are well formed and when the cercariae are free in the host's tissue spaces. Emergence of the bioculate forms also probably is due to unnatural laboratory conditions.

Large vesicular bodies, first described by Faust, 1917, are very numerous and prominent in the tail of developing forms (Fig. 19, 20, 21). These become smaller and less prominent in the tail of older developing and actively swimming forms. Kruidenier and Mehra (1957) described the distribution and character of the mucoid glands in the freshwater pronocephalid *Macrovestibulum eversum* Hsü. They found 10 pairs of irregular glands in the tail. Kruidenier, 1953, postulates the function of the body and tail gland secretions to be involved with the cuticle. No histochemical studies have as yet been carried out by me on *Cercaria P. malaclemys*. However, Fig. 21 does suggest that there is some secretion from cells within the tail. No constant number of these caudal bodies is to be found; in young developing forms the tails are packed with them (Fig. 19). I suggest that certain of these bodies may be homologous to the glycogen-containing caudal vesicles of certain cercaria including *Notocotylus* which were described by Genetzinskaja and Dobrovolski, 1962. They, therefore, logically would disappear as the actively swimming cercariae age.

Posterolateral locomotor organs are more easily seen in developing cercariae than in the emerged forms. This is probably due to the development of pigmentation in the older forms; they are extremely important in any crawling movements.

Study of the development of the excretory system indicate that it is in general agreement with that described for the Family (Kuntz, 1951). However, Figures 8, 11, 14, 15, and 16 show that there are two parts to the functional bladder. The double caudal duct, leading from the atrium as seen in developing forms dissected from rediae or lymph spaces of the snail host, cannot be observed by the

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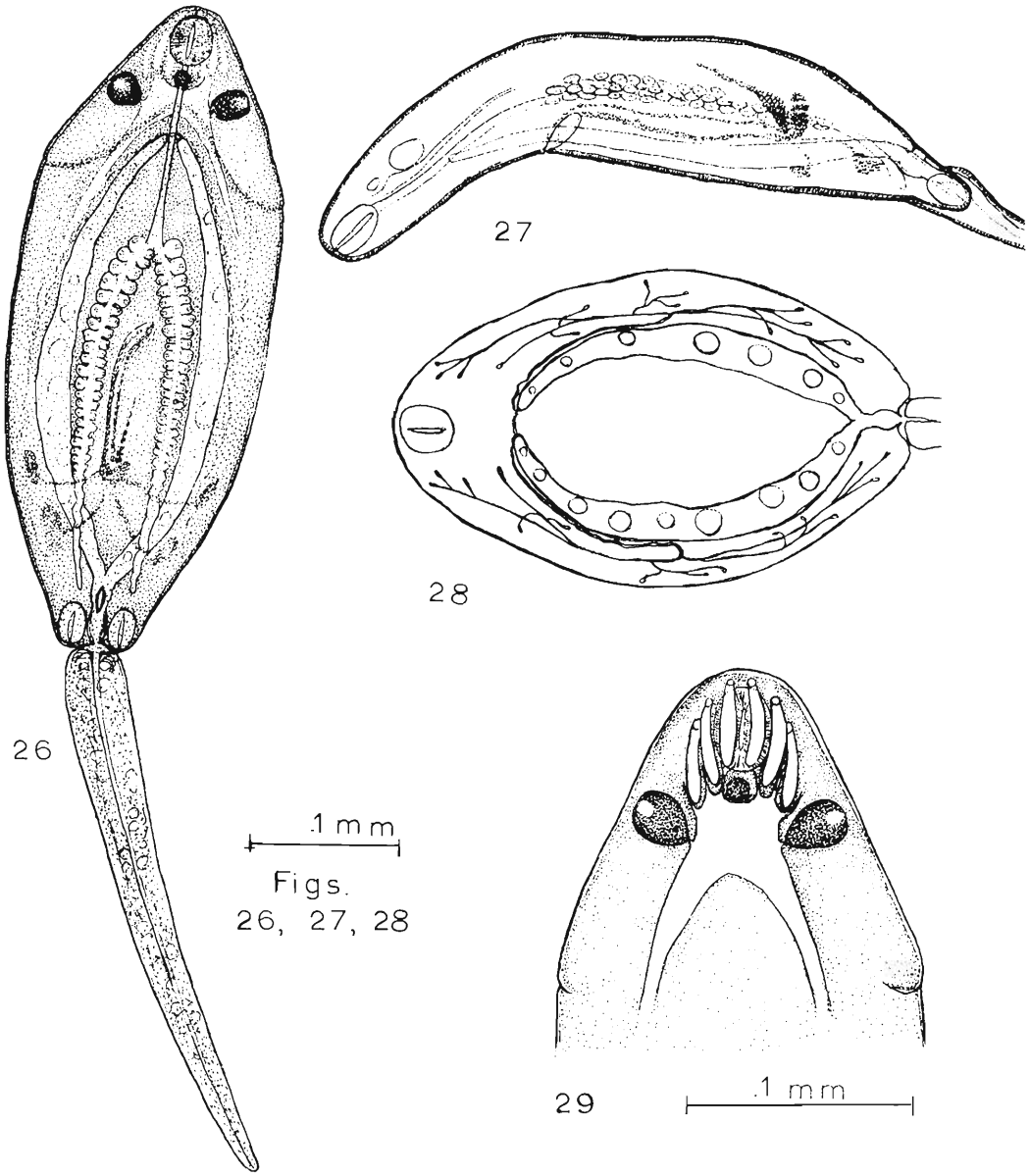
FIGS. 16–26. 16. Live, developing cercaria from snail. Collar, rudiments of reproductive organs, excretory system. (48×). 17. Live, emerged bioculate cercaria. Digestive, excretory, and reproductive systems. (100×). 18. Live, emerged trioculate cercaria. Collar and all systems evident. (48×). 19. Live, tail of young developing cercaria ex snail. Numerous vesicular bodies. (150×). 20. Live tail of older developing cercaria ex snail. Fewer vesicular bodies present. (150×). 21. Live tail of developing cercaria. Arching caudal ducts, apparent mucoid secretions around tail. (150×). 22. Live cercaria beginning to encyst. First, thin irregular layer visible. (100×). 23. Live. Metacercaria approximately 50 days old. (150×). 24. Live metacercaria dissected ex cyst. Approximately 50 days old. (150×). 25. Live adult recovered from experimentally fed and laboratory-raised *Chelonia mydas*. Ten days old. (150×). 26. Composite drawing of "mature" cercaria. Ventral view. Note primordia of reproductive system as observed in sections.




FIGS. 27–29. 27. Composite sagittal section to show relationship of digestive, excretory, and primordia of reproductive systems. 28. Schematic representation of flame-cell pattern in "mature" cercaria. 29. Detailed relationship of cephalic glands, nervous system, and three eyespots in "mature" cercaria.

time the cercariae emerge. The tubes in a developing cercaria end blindly in the mid-region of the tail (Fig. 7, 8). Aforementioned cystogenous cells and pigmentation make it very difficult to see the smaller ducts and complete flame-cell pattern. The anterior part of the cycloid duct (Fig. 26, 28) is inconspicuous indeed in this larva and only careful observations confirm its presence. Repeated observations and plotting the location of flame cells have definitely established the pattern to be that ascribed to the Family by Kuntz, 1951.

### CYST FORMATION AND METACERCARIA

Cyst formation occurs very rapidly. The cercaria settles on a suitable substrate, contracts strongly, and secretes cystogenous material from the entire body surface. While the secretion is taking place, the violently lashing tail detaches from the body; often the posterior body region contracts to form a small rounded knob between the locomotor pockets, aiding in the tail detachment. The body of the cercaria revolves actively, is contracted so as to be almost circular in outline, molding the cyst wall during its movements (Fig. 22). The cyst wall is formed in layers, the first secreted being irregular and spread out on the substrate. The completed cyst is round, dome-shaped, and firmly attached. Although a thin wall is formed within 2 to 3 min, cyst wall formation is not completed for approximately 8 to 12 min. A well-formed cyst wall averages 0.026 mm in thickness, and with age often appears yellow–brown in color. The cyst diameters exclusive of the attachment layer range from 0.315 to 0.341, 10 averaging 0.330. The diameter of the space within the cyst averages 0.294 (Fig. 23).

When cyst formation is complete, the larva relaxes, may lie in a bent or folded position; or as it ages, may shorten and lie straight across the cavity. Older metacercariae are less active than the younger, and actually show little development beyond that of the cercariae. The eyespots tend to lose their organization; remnants of them are carried over into the adult worms as dark blotches of pigment. As would be expected, the digestive system is much more visible than in the cercaria. Most of the internal organs are obscured by the natural opacity of the animal which was first described for the adult. The number of flame cells is increasing over that of the cercaria, this is evident by the spotting of an additional pair in the mid-lateral region. Pigmentation of the animals prevents following this development through to include the adult. Worms have remained viable within cysts kept in the laboratory for a period of 5 to 6 months (Fig. 24).

The larvae decrease in size with age; the average size of those dissected from month-old cysts measured 0.369 long and 0.166 wide. Measurements of the testes in a 42-day-old larva were 0.0208 by 0.0204 and 0.0194 by 0.0218; overall size of this metacercaria was not determined because of damage during dissection.

In unpublished data, Dr. John J. McDermott, Jr., found similar cercariae in *Nassarius obsoleta* from tidal ponds and marshes of the southwestern coast of New Jersey. He noted that mature cercariae are often much larger than the parent redia; this is true of my observations. The cercariae are also larger than the metacercariae which tend to decrease in size with the duration of encystment. McDermott also noted the primordia of the gonads (testes and ovary), but did not see the entire cecae nor excretory system. In spite of the failure to see the complete details, such as collar, diverticula on gut, etc., I believe that he worked with the same larva; his measurements, comparisons of the general morphology, behavior, method of encystment, the metacercaria, and the fact that many northern diamond-back terrapins were present in his collecting area permit me to name the New Jersey coast as a second locality for *Pleurogonius malaclemys*.

Detailed comparisons with other marine monostomate cercariae, e.g., *C. ephemera* Lebour, 1911, *C. lebouri* Stunkard, 1932, and *C. caribbea I* Cable, 1956, are not being given. The forms can be compared only as to the overall size and other general characteristics due to the difficulties of observing details which resulted in incomplete descriptions. *C. caribbea I* is the only one definitely described as a marine pronocephalid. Size, host differences, as well as Cable's observations on the ecological relationships and probable adult

affinities, allow me to take the liberty of assuming it to be a different species.

## FEEDING EXPERIMENTS

Although morphology of both the cercaria and metacercaria strongly indicate that the above described forms are larvae of *Pleurogonius malaclemys* Hunter, 1961, feeding experiments were undertaken to corroborate the identification. Snail opercula heavily covered with cysts were fed to the experimental hosts used. Cysts on the opercula were checked for viable metacercariae before feeding them.

Three small and immature adults were obtained from the posterior region of the small intestine of an adult female *Malaclemys t. centrata* 10 days after feeding. Forty-three mature adult worms were obtained from the same host, indicating that the experimental infection was superimposed on a natural one even though the turtle had been kept in the laboratory for 3 months prior to the infection. All turtles kept in the laboratory were fed only frozen shrimp.

Through the courtesy of Dr. Peter Klopfer of the Zoology Department of Duke University, eight 11-month-old *Chelonia mydas* which had been laboratory-raised and shrimp-fed were made available to me. These turtles change their feeding habits from carnivorous to herbivorous during their second year and using them as experimental hosts was considered questionable. However, in one of these hosts, eight immature worms were recovered 10 days after cyst-feeding. Turtles which were kept 16 to 24 days after feeding yielded no *Pleurogonius*. Besides using an unnatural host, it is very probable that the changing physiological conditions naturally occurring in them played a significant role in the results. So far, it has been impossible to obtain laboratory-raised *Malaclemys* for the experiments. However, the two successful feedings do confirm the accurate identification (Fig. 25).

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# The Effect of Gonadectomy and Hormone Therapy in Male Hamsters Upon the Egg Output by the Pseudophyllidean *Diphyllobothrium sebago**

MARVIN C. MEYER AND WILLIAM G. VALLEAU

Mammalian sex hormones have been shown experimentally to influence the burden of parasitic helminths. The female hormone generally increases the host's resistance to infection, while the male hormone typically induces a slight increase in the level of helminthiasis (Haley, 1958; Mathies, 1959; Stahl, 1961; Dobson, 1962, 1964; Miller, 1965).

Elimination of the endogenous hormone through castration has also been shown to influence the worm. Gonadectomy of mice of both sexes, infected with *Aspiculuris tetraptera*, significantly lowers the worm burden (Mathies, 1959). Solomon (1964) has shown that castration decreases the growth of *Nippostrongylus brasiliensis* in the male hamster, and that this effect can be reversed by the administration of testosterone.

Similarly, Addis (1946), working with the cyclophyllidean *Hymenolepis diminuta* in rats, showed that castration of the male causes stunting in the worm and that the growth of the worm could be returned to normal in the emasculated animal by the administration of testosterone. The egg output of *H. diminuta* also was decreased in castrate male rats, but could be restored to normal levels by the administration of testosterone (Beck, 1952). These observations indicated that normal growth and normal egg output of *H. diminuta* is directly related to the homologous sex hormone.

This report relates the effect of the male gonadal hormone on egg output by a pseudophyllidean tapeworm, *Diphyllobothrium sebago*, in male hamsters (*Mesocricetus auratus*).

## MATERIALS AND METHODS

Forty male hamsters, weighing from 80 gm to 100 gm, from Dennen Animal Industries, Gloucester, Massachusetts, were experimentally infected with *D. sebago* larvae. Each animal received three plerocercoids orally from a medicine dropper; care was taken to place the dropper well back in the oral cavity to avoid entry into the cheek pouches. Plerocercoids used in the infections were obtained from viscera of landlocked salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*) from Mooselookmeguntic Lake. The pepsin–HCl digestion technique was used in larval recovery. Larvae were transferred to 0.75% physiological saline solution, and nonmotile larvae were discarded. Host animals were maintained on an *ad libitum* diet of Purina Laboratory Chow, supplemented with lettuce leaves and water. Animals were housed individually in 23- by 23- by 40-cm metal cages with wire mesh floors. Excelsior was used as bedding.

Seven days after the hamsters had been infected, their feces were collected and examined for eggs to determine patency of the host. At 14 days postinfection the patent hosts were divided into experimental groups and one-half were anesthetized with ether and castrated. The following groups were established: (1) noncastrate untreated, (2) noncastrate treated, (3) castrate untreated, and (4) castrate treated. The administration of testosterone began on the day following castration and was given at the 1-mg level in 0.1-ml sesame oil per animal daily for 3 weeks.

Egg counts were made at 14, 21, and 35 days for each hamster by collecting the feces for a 24-hr period and soaking them overnight in a volumetric flask; the volume was then adjusted to either 100-ml or 250-ml, as necessary, and the flask was shaken vigorously. A 0.1-ml sample was then withdrawn with a micropipette from near the center of the suspension and examined microscopically, the cover glass (60 mm) having been sealed with vaseline to prevent drying and shifting of the eggs. The procedure was repeated to provide three egg counts and the mean was determined for each animal. The mean egg count for each animal, multiplied by 1,000 for the 100-ml volume and by 2,500 for the 250-ml volume,

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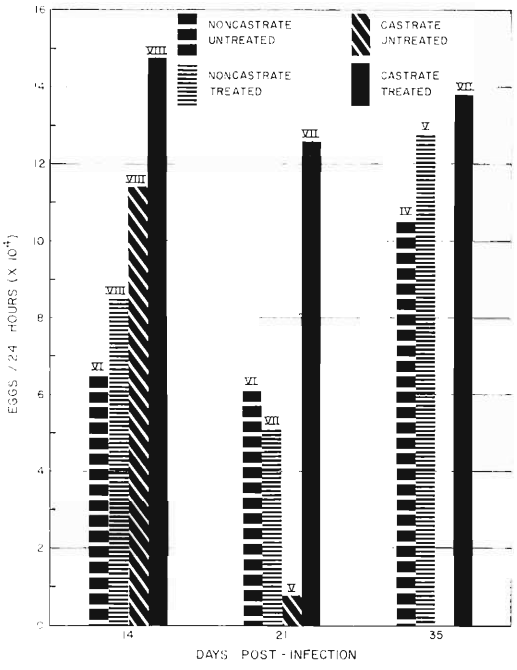


FIG. 1. Egg output of *Diphyllobothrium sebago* in noncastrate untreated and treated, and castrate untreated and treated male hamsters. The number of hosts in each group is indicated by the roman number adjacent to it.

represented the egg output for the selected 24-hr period. The mean egg counts for the different periods were expressed to the nearest thousand (Fig. 1).

Animals were sacrificed 35 days postinfection by craniocervical dislocation. At necropsy the intestine from pylorus to cecum was excised and placed atop a ruler divided into units of 1 mm. The ruler provided quick measurement of both the intestinal length and the site of scolex attachment. The worms were then transferred to saline solution, washed free of extraneous materials, and dried between paper towels; they were then measured to the nearest mm in length, and weighed on a torsion balance to the nearest mg.

## RESULTS AND DISCUSSION

All groups showed an initial decline in egg production at 21 days postinfection. Possibly this decrease in egg output coincides with loss of the primary strobila, which, in gulls, occurs at about 2 weeks postinfection (Meyer and Vik, 1963). The noncastrate untreated animals showed an increase from 61,000 eggs at day 21 to 115,000 at day 35; while treatment of the noncastrate group increased the egg output from a value of 51,000 at 21 days to 128,000 at day 35. In the untreated castrate group, the number of eggs produced 21 days postinfection was 8,000. Eggs were not detectable in the feces at 35 days after infection, despite the fact that two hosts each harbored a gravid worm at necropsy. When testosterone was administered to castrate animals, not only was the 21-day decrease in egg output reversed, but the egg production level was highest in this group throughout the period. The egg count increased from 126,000 at 21 days to 138,000 at day 35 (Fig. 1).

The wet weight of the spleen, kidneys, and adrenals did not vary significantly among the various groups. The decrease in the testes' weight of the noncastrate treated animals and the seminal vesicle stimulation is merely a reflection of effectiveness of the hormone (Table 1).

The length, weight, and point of attachment of the worms did not vary significantly among the groups. Two hamsters, one in each of the untreated groups, harbored two worms. Of the 18 positive hosts at 35 days postinfection, the remaining 16 each harbored one worm at necropsy.

Although the data presented are not extensive, they suggest that there is a relationship between *D. sebago* egg production and the male sex hormone in hamsters. These observations strengthen the conclusions of earlier workers (Addis, Beck, Mathies, Solomon) that the endogenous male sex hormone is related to helminthiasis.

## SUMMARY

The suggestion that the male sex hormone influences the egg output by *Diphyllobothrium sebago* in hamsters was examined. Fourteen days postinfection with plerocercoids one-half of the patent hosts were castrated. Four experimental groups were established: (1) noncastrate untreated, (2) noncastrate treated, (3) castrate untreated, and (4) castrate treated. Testosterone administration began on

TABLE 1. Average weights (mg) of organs from noncastrate untreated and treated, and castrate untreated and treated hamsters at necropsy. The number of animals is indicated in parentheses for each group.

| Group | Treatment | Adrenals | Spleen | Kidney | Seminal vesicle | Testes |
|---|---|---|---|---|---|---|
| Noncastrate (4) | —— | 20.1 | 66.6 | 909.8 | 309.8 | 1,604.0 |
| Noncastrate (5) | Testosterone, 1 mg × 21 days | 18.5 | 64.8 | 939.5 | 555.7 | 384.0 |
| Castrate (2) | —— | 18.2 | 64.7 | 824.6 | 42.7 | — |
| Castrate (7) | Testosterone, 1 mg × 21 days | 17.6 | 64.0 | 816.6 | 772.1 | — |

the day following castration and was given at the 1-mg level in 0.1-ml sesame oil per animal daily for 3 weeks. Egg counts were made at 14, 21, and 35 days postinfection. The castrates showed a decline in egg production throughout the 35-day experimental period, this effect was reversed by the administration of the male hormone.

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# Studies on the Life History of Two Notocotylids (Trematoda)


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## INTRODUCTION

Certain aspects of the life history of *Notocotylus stagnicolae* Herber, 1942, and *Notocotylus urbanensis* (Cort, 1914) worked out in Fort Collins, Colorado, reveal new information.

The status of certain species of *Notocotylus* has been revealed by Szidat, 1936; Harwood, 1939; Ruiz, 1946; and Dubois, 1951.

Cort (1914) described a monostome cercaria as *Cercaria urbanensis*. Harrah (1922) postulated the life history of *Notocotylus urbanensis* on the basis of morphological comparison of encysted cercariae and young adults he found infecting muskrats, *Ondatra zibethicus* (L.), a wood duck, *Aix sponsa* (L.),

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and a pintail duck, *Anas acuta* (L.). Luttermoser (1935) published an account of the life history study of *N. urbanensis* which supported Harrah's work. In this paper, he reported obtaining immature adult *Notocotylus urbanensis* with undeveloped ventral cuticular glands from a muskrat 11 days post-feeding and mature adults containing ova from a duckling 25 days after infection. He, however, expressed some doubt as to the validity of his identification of the immature parasites since they lacked the ventral glands necessary for distinguishing *Notocotylus urbanensis* from *Quinqueserialis quinqueserialis* Baker and Laughlin, 1911. Herber (1942), who worked out the life history of *Q. quinqueserialis*, stated that this parasite attains maturity in about 2 weeks. Since Luttermoser's specimens were still immature and lacked ventral glands after 11 days, it is apparent that they were not *Q. quinqueserialis* but most likely *N. urbanensis*. Harwood (1939) questioned the validity of Harrah's (1922) work and stated that "the adult form of *Cercaria urbanensis* Cort (1914) has never been properly identified, and the specific name *urbanensis* Cort, is without validity for adult trematodes until the connection has been experimentally demonstrated." In the same year, Herber (1939) reported recovering mature adult *N. urbanensis* from a muskrat on the 10th day after infection. Later, he (1955) published a fuller account of the life history of this parasite in which he, like Harwood (1939) discredited Harrah's work on the premise that Harrah "did not have material which developed from *Cercaria urbanensis*." He listed *Catatropis fimbriata* Baker, 1915 (= *Catatropis filamentis* Baker, 1915) and *Paramonostromum echinum* Harrah, 1922 as being conspecific with *N. urbanensis*. No information was given on the prepatent period of *N. urbanensis*, especially in the muskrat, the natural host which was used as one of his experimental animals.

It is the purpose of this paper to report the study made on the life history of *Notocotylus stagnicolae* and *N. urbanensis*. In addition, an attempt was made to resolve the conflicting statements made by Luttermoser and Herber concerning the time of maturity of *N. urbanensis*. New information regarding distribution and host records of the two monostomes is given.

## MATERIALS AND METHODS

Natural infection by two different species of trioculate monostome cercariae was found in the snails, *Lymnaea auricularia* (L.) collected from Dixon Lake near Fort Collins, Colorado, and *Physa gyrina* (Say) from various ponds and irrigation canals in the Fort Collins area.

Mature cercariae were studied alive both unstained and stained with intravitam dyes of Neutral Red and Nile Blue Sulfate. Photomicrographs were made of some living cercariae when possible. Cercariae were fixed in hot 10% formalin. Permanent whole mounts were studied after staining with either Mayer's acid carmine or Harris' alum hematoxylin. Cysts formed by emerged cercariae were also studied alive and as permanent whole mounts stained in Mayer's acid carmine. The specimens were cleared in beechwood creosote and mounted in piccolyte. Measurements of preserved material, unless otherwise specified, represent the average for five to 10 well extended or fairly well extended specimens. The maximum, minimum, and average for the specimens were recorded, with the average being in parentheses. Measurements of all specimens were made with the aid of an ocular micrometer and are in microns. Controlled infection experiments were conducted to obtain information on the sexually mature stages and confirm the larval identifications. Cysts formed by cercariae of *N. urbanensis* (Fig. 3) on the inside wall of containers holding *Physa gyrina* were collected in stender dishes and force-fed by means of pipette or medicine dropper to four laboratory-reared 1- to 4-day-old chicks. These chicks were fed 125, 200, and 300 cysts, respectively. Also, cysts formed from cercariae of *N. stagnicolae* which emerged from *Lymnaea auricularia* (L.) were, together with whole infected snails, fed to laboratory-reared 19- to 35-day-old ducklings, 13-day-old goslings, 19- to 35-day-old chicks, and 9-week-old albino rats. All infection experiments were conducted with cysts less than 1 day old, and our experimental animals were autopsied between 11 and 14 days after infection. Adult worms recovered were studied alive as well

TABLE 1. Comparison of 5-day-old *N. stagnicolae* and *N. urbanensis* (measurements in microns).

| | *N. stagnicolae* | *N. urbanensis* |
|---|---|---|
| Length of body | 902–1,243 (1,036.75) | 451–605 (528) |
| Width of body | 209–297 (223.5) | 84–127.2 (103.6) |
| Oral sucker | | |
| Length | 55.2–64.8 (58.6) | 38.4–45.6 (42) |
| Width | 60–72 (65.2) | 38.4–45.6 (41.2) |
| Length of esophagus | 50.4–96 (73.2) | Obscured by pigment; about 36–47 (42) |
| Ventral glands | Rudimentary; faintly seen | Undeveloped |
| No. in lateral row | About 11–12 | —— |
| No. in middle row | About 13 | —— |
| Testes | | |
| No. of lobes, lateral margin | 5–6 | Not well defined |
| Length | 121–165 (132.2) | —— |
| Width | 60–79.2 (67.2) | —— |
| Distance from posterior end | 33.6–48 (42.8) | —— |
| Ovary | | |
| Length | 48–72 (63.2) | Not well defined |
| Width (greatest part) | 40.8–60 (49.96) | —— |
| Vitellaria | Developed but not well defined; mass of small cells | Undeveloped |
| Uterine loops between vitellaria and cirrus sac | Not well defined; wavy and more advanced than in *N. urbanensis* | Not well defined; rudimentary |
| Pigmentation | Sparse and scattered | Heavily pigmented especially at the anterior part |

as when fixed in 10% formalin and stained in Mayer's acid carmine.

A comparison was made between 5-day-old *N. urbanensis* obtained from a chick and *N. stagnicolae* of the same age recovered from a duckling (See Table 1, Figs. 1, 2).

## RESULTS

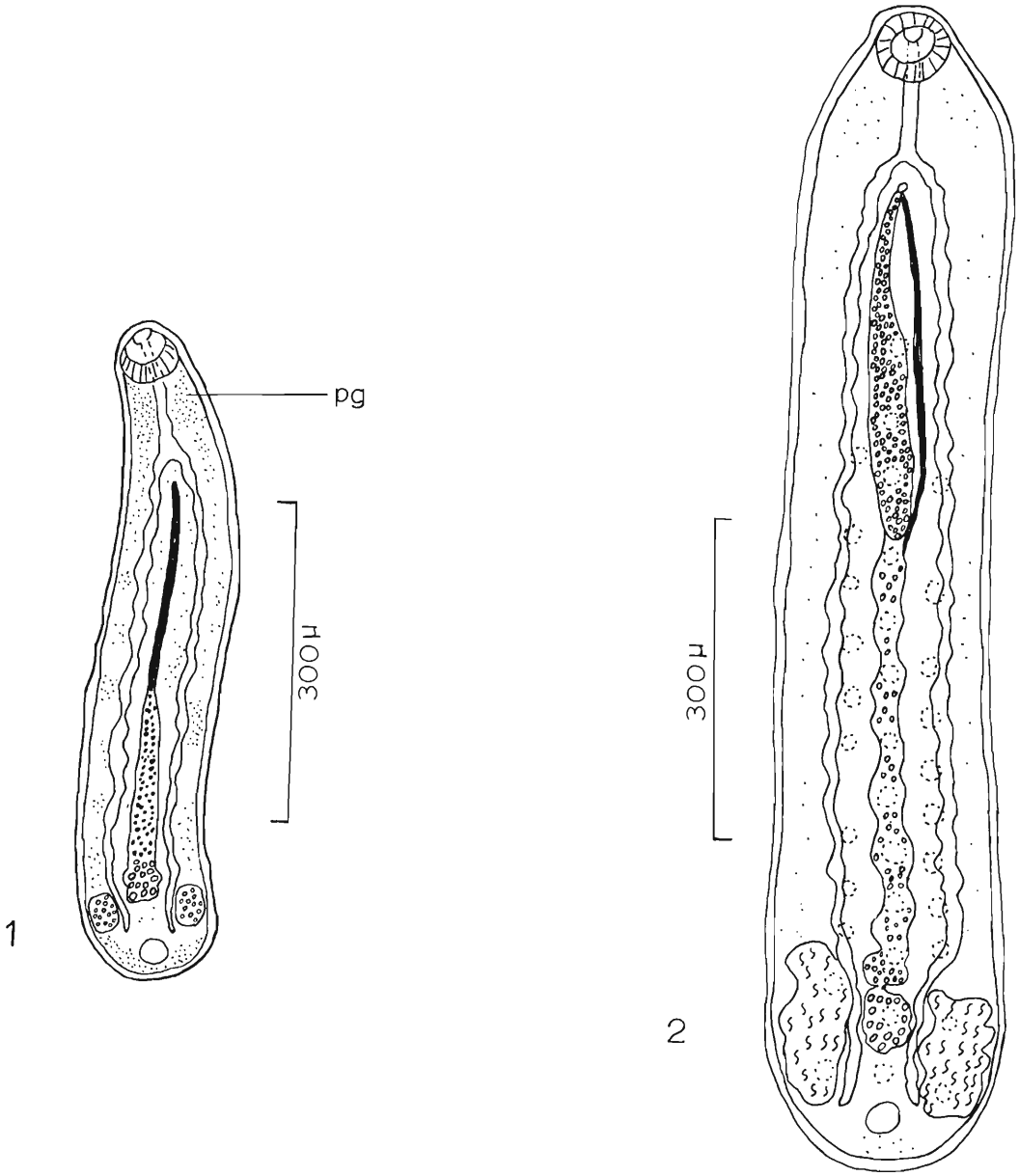
Five days postexposure, the chick fed over 300 metacercariae of *N. urbanensis* died. When autopsied, 13 preadult worms (Fig. 1) were recovered from the large intestine. On the 14th day after exposure, the remaining chicks were autopsied and found to be free of trematodes.

Mature *N. stagnicolae* were obtained from the large intestine and ceca of the experimentally infected animals. The ceca were, however, more heavily infected. The first infection was found in a duckling autopsied 5 days after administering metacercariae. Twenty-one preadult worms (Fig. 2) with rudimentary cuticular ventral glands were found. Of seven chicks exposed to infection, five were positive. The largest number of worms collected from one chick was 34. Two of three ducklings became infected. One duckling harbored 83 adult worms. Two goslings were both positive. One was infected with 38 worms. Three of four albino rats did not become infected, while one harbored a single parasite.

## DISCUSSION

Since the work of Harrah (1922) on the North American monostomes, knowledge of species of *Notocotylus* has been augmented greatly.

It is interesting to note that *N. urbanensis*, a natural parasite of muskrats, developed in a chick. Herber (1939) also reported obtaining this worm from a rooster. Luttermoser (1935) recovered it from ducklings and Kuntz (1951) from *Microtus pennsylvanicus* (Ord, 1815). In this investigation, *N. stagnicolae*, which is a parasite of ducks, developed to adulthood in chicks, ducklings, goslings, and an albino rat. These results unequivocally suggest that while the adult species of *Notocotylus* may have preferential hosts, they are non-host specific. This is a fact substantiated by Stunkard (1960) who pointed out that "host specificity of the adult stages is not definite, because the same metacercariae can mature in a number of different host species." In this respect, Herber (1942) also observed that certain of the earlier authors considered adults found in different hosts as separate species. This, according to him, is obviously unsound as one species may develop in a wide




FIGS. 1–2. 1. Five-day-old *Notocotylus urbanensis*. Dorsal view. 2. Five-day-old *Notocotylus stagnicolae*. Dorsal view.

variety of animals. He cited *N. stagnicolae* as an example of a parasite of birds that developed to maturity in mammals. Szidat and Szidat (1933), on the other hand, postulated that specificity is very marked in the case of the snail hosts. This view was supported by Herber (1942), who added that although the degree of this specificity is not known at present, it probably has real significance. The specificity of *N. urbanensis* or *N. stagnicolae* to different species of snail hosts is difficult to evaluate since it was not until 1955 that Herber demonstrated that the two species are different. In view of the close similarity of *Cercaria urbanensis* and *Cercaria stagnicolae*, all previous host records of *C. urbanensis* must be regarded with caution. However, if one assumes no misidentification by previous authors, the larval stages of the two species of *Notocotylus* show little host specificity. Cort (1914) reported finding *Cercaria urbanensis* in *Physa gyrina*; McCormick (1923) in *Goniobasis livescens* (Menke); Luttermoser (1935) in *Stagnicola emarginata angulata* (Sowerby), *Physella parkeri* (Currier), and *P. magnalacustris* (Walker, 1901); Herber (1939) in several species of *Physa* and *Stagnicola emarginata angulata*. Cercariae of *N. stagnicolae* were obtained from *Stagnicola emarginata angulata* (Sowerby) and *S. e. canadensis* (Sowerby) by Herber (1942); from *Stagnicola palustris* (Müller) by Wu (1953); and from *Lymnaea auricularia* (L.) by the present authors.

The infection experiments show that although *N. stagnicolae* is non-host specific, the adults develop more readily in birds than in mammals. Of four albino rats fed metacercariae, only one was infected, and with a single adult parasite recovered, while each of the bird species used for experimental infection sustained a fairly large number of worms. Herber (1942) fed cysts to five rats, two meadow mice, and one white mouse but recovered only one immature specimen from the white mouse and four worms from two rats. Wu (1953) got negative results from experimental infection of two white mice and two hamsters.

Table 1 shows that the preadult *N. stagnicolae* (L. 1,036 $\mu$, W. 223.5 $\mu$) is much larger than *N. urbanensis* (L. 528 $\mu$, W. 103.6 $\mu$) of the same age. While there is incipient formation of the ventral cuticular glands in *N. stagnicolae*, there is no evidence of this in *N. urbanensis*. The reproductive organs are fairly well developed in *N. stagnicolae* but undeveloped or not well defined in *N. urbanensis* (Table 1, Figs. 1, 2). The heavy pigmentation which is characteristic of the cercariae and metacercariae of *N. urbanensis* is still noticeable in this worm, especially at the anterior end, while it is sparse and scattered in *N. stagnicolae*. From this it is evident that *N. stagnicolae* at 5 days is far more advanced developmentally than *N. urbanensis* of the same age. The fact that these worms came from two different but related species of birds (chick and duckling) probably made little or no difference. The parasites are non-host spe-

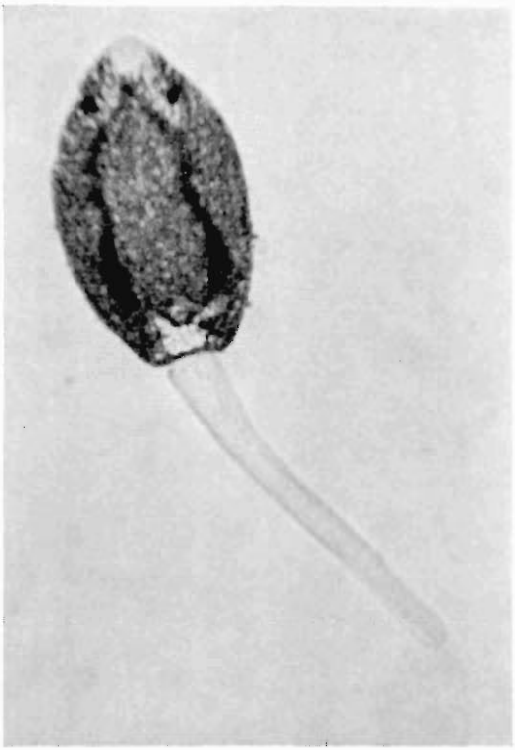


FIG. 3. Photomicrograph of living cercaria of *Notocotylus urbanensis* under cover slip pressure. Shows the three eyespots, the excretory bladder, and the excretory tubules joining above the median eyespot.

TABLE 2. Comparison of three Notocotylids (measurements in microns).

| | *N. triserialis* Diesing, 1859 (after Dubois, 1951) | *N. urbanensis* (Cort, 1914) Herber, 1955 | *N. stagnicolae* Herber, 1942 |
|---|---|---|---|
| Length of body | 1,500–2,640 | 2,670–3,620 (3,110 ± 220) | 2,670–3,400 (2,990 ± 180) |
| Width of body | 480–700 | 730–920 (800 ± 60) | 720–940 (830 ± 60) |
| Oral sucker | ? | 110–130 (120 ± 50) | 130–180 (160 ± 8) |
| Length of esophagus | 90–145 | ? | 90–180 |
| Ventral glands | | | |
| No. in lateral row | 14–16 | 15–19 (17) | 14–17 (15) |
| No. in median row | 14–15 | 13–18 (16) | 13–15 (14) |
| Testes | | | |
| No. of lobes | 5 | 2–4 | 8–12 |
| Length | 350–360 | 270–350 (320 ± 20) | 340–560 (440 ± 40) |
| Width | 170–210 | 170–230 (200 ± 20) | 170–300 (240 ± 20) |
| Distance of testes from posterior end | ? | 130–310 (210 ± 40) | 60–140 (100 ± 60) |
| Ovary | | | |
| Length | 170–180 | 160–210 (180 ± 10) | 200–300 (230 ± 20) |
| Width | ? | 130–210 (150 ± 20) | 210–300 (260 ± 20) |
| Vitellaria | ? | 17–27 groups of follicles not reaching to middle of body | 24–34 groups of follicles on each side |
| Position | Extend up to 0.52–0.57 of length of worm (0.53) | 2.1–2.5 (2.3) | 1.8–2.1 (1.9) |
| Uterine loops between vitellaria and cirrus sac on metraterm side | 3–5 | 9–13 (11) | 3–5 (4) |

cific and previous records show that matured adults of both have been recovered from either of the hosts. According to Herber (1942), *N. stagnicolae* develops to maturity in about 10 days. He (1939) recovered mature *N. urbanensis* 10 days after infection. On the basis of the present study, it seems highly improbable for *N. stagnicolae* and *N. urbanensis* to attain maturity in the same length of time. Moreover, it was pointed out by Acholonu (1964) that our specimens and those of Luttermoser (1935) agree somewhat in their rate of growth. It would appear, therefore, that the development of *N. urbanensis* to sexual maturity may be slower than reported by Herber (1939), who stated (pers. comm.) that at the time he thought the specimens from the muskrats were the result of experimental infection, and that he is no longer certain about them. He had kept the muskrat for a few weeks and then fed it some cysts. When the muskrat died, he found the specimens which he reported to have matured in 10 days; now, however, he thinks that they were from natural infections. Based on recent additional work on this species, he (Herber, 1964) added in his communication that *N. urbanensis* matures in laboratory rats and *Microtus* in about 24 days, which is a day shorter than the time reported by Luttermoser (1935). This statement is apparently more accurate than that of 1939 and verifies the work of Luttermoser.

Dubois (1951) considered *N. urbanensis* (Cort, 1914), Harrah (1922) *ex parte* and *N. stagnicolae* Herber, 1942, as synonyms of *N. triserialis* Diesing, 1839. Available information shows that this synonomy is not valid.

1. Dubois' (1951) synonymy is based on the *N. urbanensis* collected by A. Hassall in Maryland, USNM: No. 5772, No. 5771, No. 5769, and No. 5770, upon which Harrah's (1922) work was based and about which Herber (1955) wrote ". . . it seems justifiable to conclude that Harrah did not have material which developed from *Cercaria urbanensis*, and that, therefore, his description of *Noto-*

TABLE 3. Comparison of the Larval forms of three Notocotylids (measurements in microns).

| | *Cercaria vaga* Szidat and Szidat, 1933 | *Cercaria* of *N. urbanensis* (Cort, 1914) (after Herber, 1955) | *Cercaria* of *N. stagnicolae* Herber, 1942 |
|---|---|---|---|
| Body of cercaria | | | |
| Length | 544 | 351–406 (383) | 320–570 |
| Width | 152 | 140–187 (161) | 80–210 |
| Tail | 600 | 671–764 (721) | 620–940 |
| Excretory tubules | Join anteriorly caudad to median eyespot | Join anteriorly ahead of median eyespot | Join anteriorly caudad to median eyespot |
| Time of encystment | ? | Very soon after emergence; usually within 3–5 min | Delayed: 5–20 min |
| Cyst size | 230 | 220–260 | 200–250 |
| Mother sporocyst | ? | Contain 4–8 mother redia | Contain only one mother redia |
| Mother/redia | Only daughter redia figured but measurement not given | 308–871/81–308 | 170–1,330/80–190 |

*cotylus urbanensis* must be discarded." Harwood (1939) previously expressed this same notion. He stated that "the adult form of *Cercaria urbanensis* Cort, 1914 is without validity for adult trematodes until the connection has been experimentally demonstrated." Herber (1955) (4 years after Dubois' work) experimentally demonstrated this connection and thus established *N. urbanensis* as a separate and valid species.

2. The life history of *N. stagnicolae* and *N. urbanensis* conducted by Herber (1942, 1955) and restudied by the present authors shows that these two notocotylids are not identical (see Table 1). Also, a comparative examination of these two and the type species, *N. triserialis*, as described by Dubois (1951), reveals that the three are seemingly separate and different species (see Tables 2 and 3). While this comparison brings out some similarities in these three species, in our opinion, the dissimilarities are enough to warrant their consideration as separate species. We are in agreement with Herber (1955), who gave the following diagnostic characters as being helpful in separating species of the genus *Notocotylus*: (1) the anterior extent of the vitellaria, (2) the lateral lobation of the testes, (3) body and tail size of cercariae, (4) the course of the excretory tubules of the cercariae, and (5) cyst size. Tables 2 and 3 show that these worms differ, *inter alia*, with respect to numbers 1, 2, and 4.

Stunkard (1960) stated that the determination of species of *Notocotylus* is difficult. The worms, according to him, are very similar and

TABLE 4. Species of Notocotylus with their life history worked out.

| Species | Cercaria | Intermediate host(s) |
|---|---|---|
| 1. *N. stagnicolae* Herber, 1942 | Cercaria of *N. stagnicolae* Herber, 1942 | Generally in *Stagnicolae* spp.; *Lymanaea auricularia* (L.) |
| 2. *N. urbanensis* (Cort, 1914), Herber, 1955<br>Syn. *Paramonostomum echinum* Harrah, 1922; *Catatropis fimbriata* Barker, 1915<br>*C. filamentis* Barker, 1915 | *Cercaria urbanensis* Cort, 1914 | Generally in *Physa* spp. *Goniobasis livescens* (Menke), *Stagnicola emaginata angulata* (Sowerby) |
| 3. *N. triserialis* Diesing, 1839 | *Cercaria vaga*[1] Szidat and Szidat, 1933 | *Lymnaea palustris* (Müll.) |
| 4. *N. ephemera* (Nitzsch, 1807) (= *N. thienemanni*) Szidat and Szidat, 1933 | *Cercaria ephemera* Nitzsch, 1807 | *Planorbis corneus* (L.) |
| 5. *N. imbricatus* Szidat, 1934 | *Cercaria imbricata* Looss, 1893 | *Bithynia tentaculata* (L.) |
| 6. *N. minutus* Stunkard, 1960 | Cercaria of *N. minutus* Stunkard, 1960 | *Hydrobia minuta* (Totten) |
| 7. *N. seineti* Fuhrmann, 1919 | *Cercaria monostomi* Looss? (after Szidat, 1936) | ? |

[1] Described as the cercaria of *N. attenuatus* Rud. 1809 by Szidat and Szidat, 1933. (See Dubois, 1951: 54.)

specific differences in morphology are small. Such being the case, the separation of closely related species and the establishment of synonymous ones (which so far is a point of much confusion) could better be accomplished through life history studies. Of all known species of *Notocotylus* (20 listed by Dubois (1951)) plus *N. stagnicolae* Herber, 1942, *N. urbanensis* (Cort, 1914) Herber, 1955, and *N. minutus* Stunkard, 1960, only six or seven have their life cycles elucidated (see Table 4). Thus, the necessity of resolving the life history of more of the described species of the genus *Notocotylus* cannot be overemphasized.

As far as the authors are aware, this is the first record of *N. urbanensis* and *N. stagnicolae* from Colorado. The finding of *N. stagnicolae* in *Lymnaea auricularia* (L.) represents a new host record. The goose is also a new experimental definitive host for *N. stagnicolae*.

## SUMMARY

A study was made of certain aspects of the life history of *Notocotylus stagnicolae* Herber, 1942 and *N. urbanensis* (Cort, 1914).

An attempt was made to resolve the conflicting statements made by Luttermoser (1935) and Herber (1939) concerning the time of maturity of *N. urbanensis*. This study backed by Herber's (1964) work on *N. urbanensis*, and his personal communication, verifies Luttermoser's work.

Available information from life history studies shows that *N. urbanensis* and *N. stagnicolae*, considered as synonyms of *N. triserialis*, Diesing, 1839, by Dubois (1951), are separate and valid species.

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# *Paraphelenchus acontioides* n. sp. (Nematoda: Paraphelenchidae), a Mycophagous Nematode from Illinois, with Observations on its Feeding Habits and a Key to the Species of *Paraphelenchus*

D. P. TAYLOR AND J. K. PILLAI*

An undescribed species of the genus *Paraphelenchus* (Micoletzky, 1922) Micoletzky, 1925 was recovered from soil collected around the roots of Kentucky blue grass (*Agrostis palustris* Huds.) on the Horticulture Research Farm at the University of Illinois, Urbana. This species was established in 1960 on the fungus *Pyrenochaeta terrestris* (Hansen) Gorenz., J. C. Walker, and Larson, and has been maintained in the laboratory since that time.

*Paraphelenchus acontioides* n. sp.

DIMENSION: Females (n = 60): L = 0.77 ± 0.04 mm (0.71–0.88 mm); a = 28.5 ± 1.4 (25–31); b = 4.8 ± 0.2 (4.4–5.3); c = 25 ± 1.5 (20–30); V = 74.8 ± 0.7 (73–77); Stylet = 15 ± 1 μ (14–16 μ).

DESCRIPTION: Body slightly arcuate ventrally when heat-relaxed; gradually tapers anteriorly to a low rounded lip region which is continuous with the body contour. Cuticle marked by fine transverse striae, approximately 1 μ apart. Lateral field occupies one-fourth to one-fifth body width and contains eight incisures throughout most of its length. Number of incisures reduced to six in the isthmus region, lateral field fading out anterior to corpus. Incisures also reduced to six in anal region, becoming indistinct near the tail tip. Body tapers posteriorly to a short tail, less than twice anal body diameter. Dorsal surface of the tail curved more than the rather straight ventral surface. Tail tip with a single mucro as in Figure 1 F, G, and H.

Lip region not striated, consisting of six lips. Neither amphids nor papillae seen. Spear guiding ring a circumoral circle composed of six short pieces fused together (Fig. 1C). Stylet 14–16 μ long with a shorter conical anterior portion and a cylindrical shaft. Stylet has slight basal swellings which are more pronounced on the ventral side. Procorpus slender with conspicuous lumen. Metacorpus large, aphelenchoid, with valvular apparatus slightly posterior to the center. Esophageal gland ducts open into metacorpal lumen in typically aphelenchoid arrangement. Length of the lumen between the openings of the glands and the beginning of the valve is highly sclerotized. Isthmus slender, gradually widening to form basal bulb. Three esophageal glands confined within basal bulb. Lumen of isthmus and basal bulb less sclerotized than that of corpus. Intestine composed of two rows of easily discernible cells, usually filled with refractive globules in specimens from active cultures. Intestinal lumen forming a wide anterior chamber, becoming narrower and sinuous throughout the rest of its length. Rectum about one and one-half times the anal body diameter. Anal opening about a third the body diameter at that point as in Figure 1F. Nerve ring surrounding the isthmus immediately behind metacorpus. Dierids papillate, conspicuous, located in the lateral field at the level of the nerve ring. Excretory pore opposite nerve ring with canal leading ventrally to a unicellular gland ventral to the junction of esophagus and intestine. Hemizonid just posterior to the excretory pore; hemizonion one body width posterior to it. Phasmids located near the tail terminus appearing as minute papillae each with a fine strand connected to the body content.

Ovary single, outstretched. Oocytes arranged in a single row surrounded by a conspicuous epithelium. Epithelial cells with nuclei slightly larger than in oocytes. Anterior portion of epithelium illustrated in Figure 1A; posterior portion omitted for clarity. Uterus composed of paired ovoid cells ranging from 7–13 in number. Post vulvar sac about twice the vulval body width in length. Vulva a transverse slit with conspicuous vulvar lips, observable in young females prior to escape from fourth stage cuticle. One or two eggs

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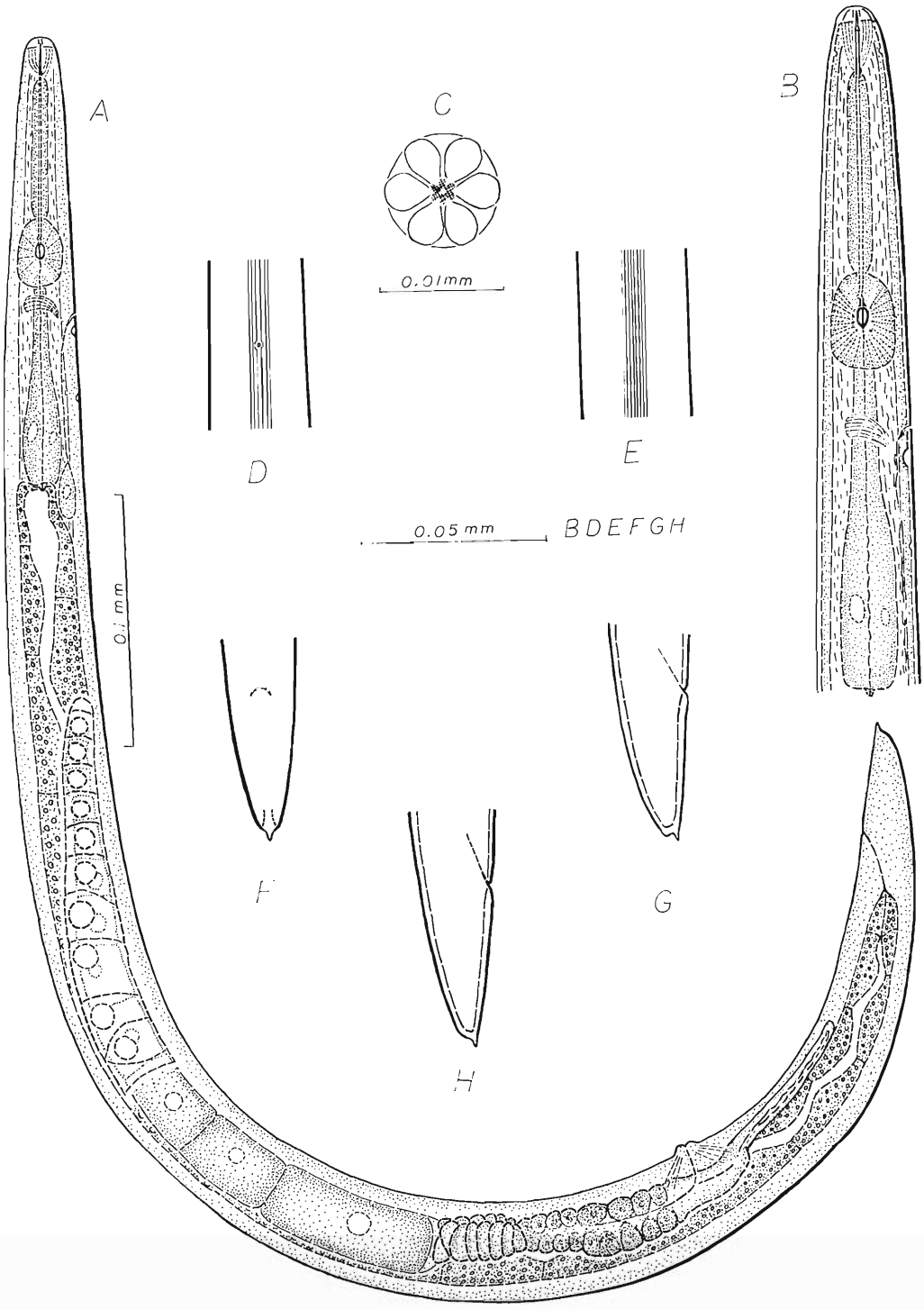

may be present at a time in egg-laying females. Eggs laid in the single celled stage, measuring 64–74 $\mu$ in length and 28–32 $\mu$ in width.

HOLOTYPE: Female, progeny of a female collected in 1960 and maintained on cultures of *Pyrenochaeta terrestris* growing on Potato Dextrose Agar, slide # P-H-1, Nematode Slide Collection, Department of Plant Pathology, University of Illinois, Urbana.

PARATYPES: Ten females, date same as for holotype, slide # P-P-1, Nematode Slide Collection, Department of Plant Pathology, University of Illinois, Urbana.

TYPE HABITAT AND LOCALITY: Soil around roots of Kentucky blue grass (*Agrostis palustris* Huds.), Horticulture Research Farm, University of Illinois, Urbana.

DIAGNOSIS: *Paraphelenchus acontioides* n. sp. can be distinguished by the presence of a mucro on the tail tip from *P. batavicus* Filipjev, 1934, *P. myceliophthorus* J. B. Goodey, 1958, and *P. tritici* Baranovskaya, 1958, all of which lack such a terminal process. Each of those species possessing mucrones has a characteristic number of lines in the lateral field: *P. basili* Das, 1960 has four; *P. amblyurus* Steiner, 1934 has six; nine were illustrated in *P. pseudoparietinus* Micoletzky, 1922; whereas *P. acontioides* n. sp. has eight. The new species also differs from *P. pseudoparietinus*, its closest relative, in that the lip region in the former is continuous with the body contour and males are not known, whereas the lip region in the latter is offset and males are known.

### KEY TO THE SPECIES OF THE GENUS *Paraphelenchus*

1. Tail tip with one or more mucrones ..... 2
1. Tail tip without mucro ..... 5
   2. Tail tip tapering evenly dorsally and ventrally to a blunt terminus, lateral field with 4–6 lines ..... 3
   2. Tail tip with prominent curvature on the dorsal surface, lateral field with 8–9 lines ..... 4
3. Lateral field with 4 lines, lip region offset ..... *P. basili* Das, 1960
3. Lateral field with 6 lines, lip region continuous with the body contour ..... *P. amblyurus* Steiner, 1934
   4. Lateral field with 8 lines, lip region continuous with the body contour ..... *P. acontioides* n. sp.
   4. Lateral field with 9 lines, lip region offset ..... *P. pseudoparietinus* (Micoletzky, 1922) Micoletsky, 1925
5. Spicules of males with a sharp ventral process, manubrium more than half the length of the shaft ..... 6
5. Spicules of males without a ventral process, manubrium less than half the length of the shaft ..... *P. batavicus* Filipjev, 1934
   6. Female tail with a pair of subterminal papillae, lip region continuous with the body contour ..... *P. myceliophthorus* J. B. Goodey, 1958
   6. Female tail without any tail process, lip region offset ..... *P. tritici* Baranovskaya, 1958

### FEEDING HABITS OF *P. acontioides*

Observations were made using nematodes which had been transferred from stock cultures to 4-day-old fungus cultures growing in a very thin layer of 2% water agar in petri dishes. After a cover slip was placed over them it was possible to observe details with a 90× oil immersion lens. Feeding habits described here are based on observations made while the nematodes were feeding on the fungus, *Pyrenochaeta terrestris*.

This nematode is a voracious feeder, emptying a cell within a few seconds and then moving to the next cell in a very short time. The same general area of feeding was sometimes used for more than an hour during which the posterior half of the body remained at the same place, with only occasional movements, while the anterior half moved in various directions to feed on cells within its reach.

Movement of *P. acontioides* was of the usual type with the head turning to the sides accom-

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FIG. 1. *Paraphelenchus acontioides* n. sp., A, Female; B, anterior region; C, *en face* view; D, lateral field near the isthmus; E, lateral field near center of body; F, ventral view of tail; G and H, lateral view of tail tip, H-typical, and G-a variant.

panied by occasional protrusion of the stylet. Once the head touched a hypha, the nematode stopped and fed on it, or bypassed it. When a cell was chosen for feeding the nematode thrust its stylet into the cell with the head appressed to the cell wall. The number of stylet thrusts usually varied from 10–15 at a rate of 15–20 thrusts per second; but in some cases penetration was achieved with only 4–8 stylet thrusts. Number of stylet thrusts required for penetration could not be correlated with the proximity of the septum, nature of the cell wall, or the turgidity of the cell. The action of the stylet was always associated with a twitching movement at the esophagus-intestine junction. This movement was also observed during the metacorpal pumping and esophago-intestinal valve action. Once the stylet gained entry into the cell there was a short pause, lasting only a fraction of a second, and soon the metacorpal pump action started. After five to eight pumpings at a rate of about four per second the nematode left the cell. As a rule this nematode withdrew all the cell contents. When pumped into the intestine the cell contents formed a jelly-like mass that was retained in the transparent anterior chamber until the next feeding when it was pushed down as such into the lumen. As the food passed along the lumen of the intestine it decreased in bulk suggesting digestion and assimilation were occurring. The jelly-like mass moved back and forth in the intestine with body movements. Even after continuous feeding for 30 minutes there were not more than four or five such jelly masses in the length of the lumen suggesting that the rate of digestion was fast enough to keep up with the ingestion, though the nematodes were voracious feeders.

The feeding process took 2 to 3 seconds; 1 second for stylet insertion followed by 1 to 2 seconds of metacorpal pump action. The action of the metacorpal bulb allowed the withdrawal of the cell contents and presumably also aided the flow of saliva into the esophageal lumen. Thus, food was mixed with the saliva before it reached the intestine.

Feeding habits of an isolate of *Aphelenchus avenae* Bastian, 1865 were studied for comparison and it differed in the following details. *A. avenae* usually required about 20–50 stylet thrusts for penetration of a cell wall, whereas *P. acontioides* required only 10–15 stylet thrusts. The twitching movement associated with the stylet action was in the upper quarter of the median bulb in *A. avenae*, whereas *P. acontioides* exhibited a twitching movement at the esophago-intestinal junction and not at the median bulb. As a rule *P. acontioides* withdrew all the cell content from the cell, whereas *A. avenae* frequently left the cell partially emptied. In *A. avenae* the cell contents appeared as globules when pumped into the intestinal lumen, and not as a jelly-like cytoplasmic mass. The feeding habits of both the species were similar in other details.

## SUMMARY

*Paraphelenchus acontioides* n. sp., recovered from soil samples around the roots of Kentucky blue grass (*Agrostis palustris* Huds.) is described, its feeding habits compared with those of *Aphelenchus avenae*, and a key to the species of the genus *Paraphelenchus* is given.

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# Four New Species of *Gyrodactylus* (Trematoda: Monogenea) from Southeastern U. S.*

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The host specimens used in this study were collected by seine and with HTH (calcium hypochlorite). The *Gyrodactylus* species were collected using the method described by Putz and Hoffman (1963), and were treated and measured as described by Rogers and Wellborn (1965).

The species described in this paper were collected as part of a survey of fish parasites of National Fish Hatcheries.

All measurements are in microns. Averages are to the nearest micron and ranges are given in parentheses. The illustrations were drawn with the aid of a camera lucida.

## *Gyrodactylus bretinae* sp. n.
(Figs. 1–5)

HOST AND LOCALITY: *Etheostoma stigmaeum* (Jordan) speckled darter, National Fish Hatchery, Corning, Clay County, Arkansas.

LOCATION ON HOST: Fins and body.

SPECIMENS STUDIED: Eleven.

TYPE SPECIMEN: Type and one paratype USNM Helm. Coll. No. 61626 and 61627. Paratypes in author's collection.

DESCRIPTION: Medium-sized gyrodactylid with fusiform body and smooth thin cuticle; greatest length 450 (340 to 490), greatest width 104 (90 to 120). Haptor umbrella-shaped, 97 long by 105 wide, with 16 marginal hooks. Anchors moderate, 64 (62 to 67) from tip of base to most distant points of curvature (Fig. 1); length of point 27 (25 to 30). Length of dorsal bar 23 (19 to 27), width at center 3 to 4; ends of bar slightly constricted then expanded into terminal plates 9 to 10 long by 5 (4 to 6) wide; center of dorsal bar with small simple notch on posterior edge (Fig. 2). Length of ventral bar 27 (24 to 29); width at center 6 (5 to 7); with large, bluntly rounded lateral anterior projections that extend out from bar at approximately a 45° angle (Fig. 3); length 10 (8 to 11) measured to anterior edge of ventral bar. Shield of ventral bar 17 (15 to 20) long; width at proximal end 18 (16 to 23); with lateral margins tapering to concave distal end. Length of marginal hooks 7 to 8 from tip of bilobed base to most distant point of curvature (Fig. 4); shaft 30 (28 to 32) long; lamella U-shaped, 11 (10 to 13) long. Cirrus pouch 13 (12 to 16) in diameter, on left of ventral midline just posterior to pharynx. Cirrus with one large spine and four prominent stylets (Fig. 5). Pharynx 46 (37 to 50) in transverse diameter; bilobed in side view with both lobes approximately same diameter. Each cephalic lobe with a prominent spine that protrudes a distance of 4 to 5.

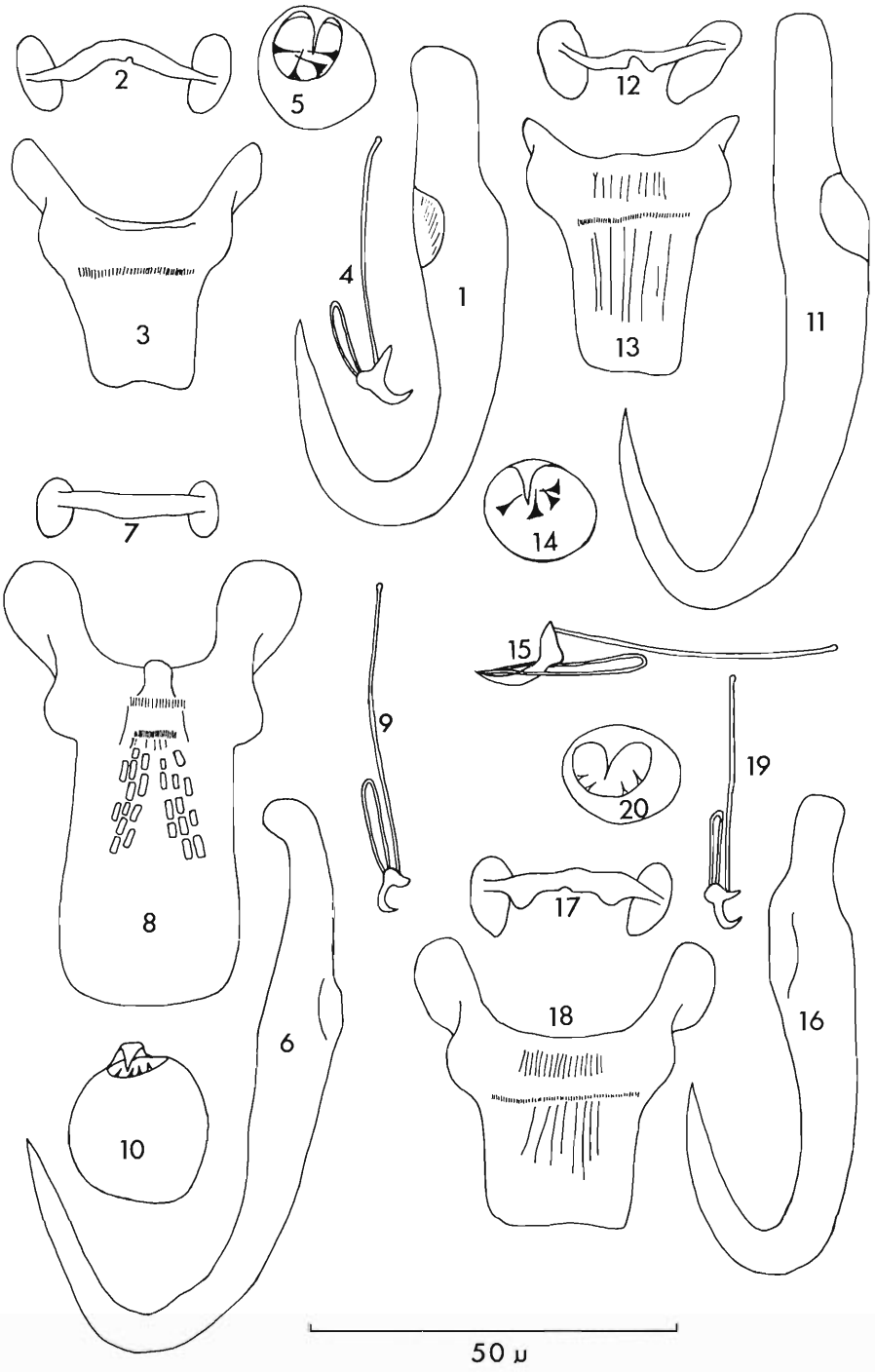
COMPARISON: *Gyrodactylus bretinae* resembles *G. eucaliae* Ikezaki and Hoffman, 1957, *G. heterodactylus* Rogers and Wellborn, 1965, *G. macrochiri* Hoffman and Putz, 1964, and *G. percinae* Rogers and Wellborn, 1965, by the presence of a notch in the dorsal bar. *G. percinae* and *G. macrochiri* have a large C-shaped notch in the dorsal bar (fig. 24, Rogers and Wellborn, 1965; fig. 1, Hoffman and Putz, 1964), whereas *G. bretinae* has a small simple notch in the dorsal bar. It differs from *G. heterodactylus* by having marginal hooks that are all equal in size. *G. eucaliae* differs by having a sickle-shaped marginal hook that lacks a lamella, and a cirrus that possesses eight small stylets.

## *Gyrodactylus campostomae* sp. n.
(Figs. 6–10)

HOST AND LOCALITY: *Campostoma anomalum* (Rafinesque), stoneroller, Chewacla Creek, Lee County, Alabama.

ADDITIONAL LOCALITIES: Elkhorn Creek, National Fish Hatchery, Frankfort, Franklin County, Kentucky.

* This research was partially supported by the Southeastern Cooperative Fish Parasite and Disease Project and carried out in facilities provided by Auburn University, Auburn, Alabama.



LOCATION ON HOST: Fins and body.

SPECIMENS STUDIED: Twelve.

TYPE SPECIMEN: Type and one paratype USNM Helm. Coll. No. 61628 and 61629. Paratypes in author's collection.

DESCRIPTION: Medium-sized gyrodactylid with fusiform body and smooth thin cuticle; greatest length 421 (360 to 480), greatest width 72 (55 to 80). Haptor inverted saucer-shaped, 95 (91 to 100) long by 98 (95 to 100) wide; with 16 marginal hooklets. Anchors large, 78 (74 to 80) from tip of base to point of greatest curvature; length of point 30 (25 to 33). Tip of anchor base bent toward point of anchor (Fig. 6). Length of dorsal bar 22 (18 to 25), width at center 3 to 4; ends of dorsal bar expanded into small oval terminal plates, 5 to 6 long by 4 to 5 wide (Fig. 7); dorsal bar of almost uniform width except for terminal plates. Length of ventral bar 26 (24 to 30), width at center 9 (8 to 10); with massive, bluntly rounded lateral anterior projections that extend out from ventral bar at approximately an 80° to 90° angle; length 15 (14 to 17) measured to anterior edge of ventral bar (Fig. 8). Ventral bar with a large medial knob 5 to 6 long by 4 to 5 wide. Shield of ventral bar 28 (25 to 30) long; width at proximal end 17 (15 to 20); with lateral margins tapering slightly outwards to truncated distal end. Ventral shield with six rows of rectangular, refractile ridges that are divided into two groups of three parallel rows each. Length of marginal hook 5 to 6 from tip of slightly bilobed base to most distant point of curvature; lamella U-shaped, 14 (13 to 15) long and difficult to see except with phase contrast microscope; shaft 39 (36 to 41) long. Cirrus pouch 18 (15 to 19) in diameter, on left of ventral midline just posterior to pharynx. Cirrus with one large spine and four to five stylets. Pharynx 31 (25 to 35) in transverse diameter; bilobed in side view with both lobes approximately same diameter. Each cephalic lobe with a spine that protrudes a distance of 2 to 3.

COMPARISONS: *Gyrodactylus campostomae* most closely resembles *G. rhinichthius* Wood and Mizelle, 1957, and *G. protuberus* Rogers and Wellborn, 1965, by possessing a medial knob on the ventral bar. It differs from both *G. rhinichthius* and *G. protuberus* by having much larger anchors, larger lateral anterior projections, and the shield of the ventral bar and the dorsal bar are of a different shape.

### *Gyrodactylus lineadactylus* sp. n.
(Figs. 11–15)

HOST AND LOCALITY: *Promoxis nigromaculatus* (LeSueur), black crappie, Oktibaha Creek, National Fish Hatchery, Meridian, Lauderdale County, Mississippi.

LOCATION ON HOST: Body and fins.

SPECIMENS STUDIED: Seventeen.

TYPE SPECIMEN: Type and one paratype USNM Helm. Coll. No. 61630 and 61631. Paratypes in author's collection.

DESCRIPTION: Medium-sized gyrodactylid with fusiform body and smooth thin cuticle; greatest length 478 (400 to 520), greatest width 86 (80 to 95). Haptor umbrella-shaped, 90 long by 100 wide. Anchors large, 78 (76 to 83) from tip of base to most distant point of curvature; length of point 29 (25 to 31) (Fig. 11). Length of dorsal bar 27 (24 to 29), width at center 3 to 4; ends of bar constricted slightly then expanded into terminal plates 10 to 11 long by 5 to 6 wide; center of dorsal bar with large simple notch on posterior edge (Fig. 12). Length of ventral bar 27 (26 to 29), width at center 8 to 9; with small sharply pointed lateral anterior projections that extend out from bar at approximately a 45° angle (Fig. 13); length 5 (4 to 6) measured to anterior edge of ventral bar. Shield of ventral bar 21 (20 to 24) long; width at proximal end 15 to 16; lateral margins almost parallel, distal end slightly concave; with

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FIGS. 1–20. New species of *Gyrodactylus* from the Southeastern U. S. Figs. 1–5. *Gyrodactylus bretinae* sp. n. 1, anchor; 2, dorsal bar; 3, ventral bar and shield; 4, marginal hook; 5, cirrus. Figs. 6–10. *G. campostomae* sp. n. 6, anchor; 7, dorsal bar; 8, ventral bar and shield; 9, marginal hook; 10, cirrus. Figs. 11–15. *G. lineadactylus* sp. n. 11, anchor; 12, dorsal bar; 13, ventral bar and shield; 14, cirrus; 15, marginal hook. Figs. 16–20. *G. minytremae* sp. n. 16, anchor; 17, dorsal bar; 18, ventral bar and shield; 19, marginal hook; 20, cirrus.

seven to 10 fine striations. Length of marginal hook 11 (10 to 12) from tip of base to most distant point of curvature; lamella U-shaped, 21 (19 to 23) long; shaft 31 (29 to 32) long. Marginal hook only slightly recurved and base almost straight. Lamellae appear to be attached to tip of point of marginal hook; shaft of marginal hook passes through arms of lamella (Fig. 15). Cirrus pouch 13 to 15 in diameter, on left of ventral midline just posterior to pharynx. Cirrus with one large spine and four prominent stylets (Fig. 14). Pharynx 37 (35 to 40) in transverse diameter; bilobed in side view. Spines did not protrude from cephalic lobes in specimens studied.

COMPARISON: *Gyrodactylus lineadactylus* may be distinguished from other North American gyrodactylids by the long lamellae attached to the tip of the point of the slightly recurved marginal hooks. Also it is unique in that the shaft of the marginal hook passes through the arms of the lamella.

### *Gyrodactylus minytremae* sp. n.
(Figs. 16–20)

HOST AND LOCALITY: *Minytrema melanops* (Rafinesque), spotted sucker, Chewacla Creek, Lee County, Alabama.

LOCATION ON HOST: Fins and body.

SPECIMENS STUDIED: Nine.

TYPE SPECIMEN: Type and paratype USNM Helm. Coll. No. 61632 and 61633. Paratypes in author's collection.

DESCRIPTION: Medium-sized gyrodactylid with fusiform body and smooth thin cuticle; greatest length 520 (490 to 570), greatest width 81 (70 to 90). Haptor umbrella-shaped, 93 (80 to 110) long by 98 (85 to 120) wide. Anchors 63 (61 to 65) from tip of base to most distant point of curvature; length of point 25 (23 to 27) (Fig. 16). Length of dorsal bar 25 (23 to 28), width at center 3 to 4: ends constricted then expand into terminal plates 9 to 10 long by 5 wide; bar with prominent protrusions on posterior edge just medial to constrictions. Dorsal bar with shallow simple notch in center of posterior edge (Fig. 17). Length of ventral bar 30 (28 to 31), width at center 7 (6 to 8); with large, bluntly rounded lateral anterior projections, 14 (13 to 16) in length measured to anterior edge of ventral bar (Fig. 18); lateral anterior projections extend out from dorsal bar at approximately a 50° to 60° angle. Shield of ventral bar 19 (17 to 20) long; width at proximal end 23 (21 to 25); with lateral margins almost parallel; distal end slightly concave; with seven to 10 fine longitudinal striations. Length of marginal hooks 5 to 6 from tip of bilobed base to most distant point of curvature; shaft 26 (25 to 28) long; lamella U-shaped, 10 to 11 long (Fig. 19). Cirrus pouch 13 (12 to 15) in diameter, on left of ventral midline just posterior to pharynx. Cirrus with one large spine and four to five small stylets. Pharynx 38 (35 to 40) in transverse diameter; bilobed in side view with both lobes of approximately same diameter. Cephalic lobes with a prominent spine that protrudes a distance of 5 to 6.

COMPARISONS: *Gyrodactylus minytremae* most closely resembles *G. eucaliae* Ikezaki and Hoffman, 1957, *G. bretinae*, and *G. lineadactylus* on the basis of the dorsal bar possessing a simple notch on the posterior edge, but differs from them in the shape and size of the anchors, the size and shape of the ventral bar, and in the shape of the dorsal bar.

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## SUMMARY

Four new species of *Gyrodactylus* are described from freshwater fishes of the Southeastern U. S.: *G. bretinae* from *Etheostoma stigmaeum* (Jordan), *G. campostomae* from *Campostoma anomalum* (Rafinesque), *G. lineadactylus* from *Pomoxis nigromaculatus* (LeSueur), and *G. minytremae* from *Minytrema melanops* (Rafinesque). All fish hosts were collected from Alabama, Arkansas, Kentucky, and Mississippi.

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## Cultivation of the Turkey Coccidium, *Eimeria meleagrimitis* Tyzzer, 1929, in Mammalian Kidney Cell Cultures

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Long (1965) obtained complete development of the chicken coccidium, *Eimeria tenella*, from sporozoites to oocysts in the chorioallantoic membrane of embryonated eggs. Patton (1965), however, was the first to obtain development of a coccidium in cell culture. Utilizing monolayer cultures of bovine kidney cells (secondary, cell line) and fibroblast-like cells (secondary) derived from 9-day-embryonated Japanese quail, he obtained development of *E. tenella* sporozoites to mature, first generation schizonts. His was the first report of cultivation of an obligate intracellular intestinal protozoan parasite in cell culture. Strout *et al.* (1965) have reported establishment of *E. acervulina* sporozoites in monolayer cultures of chick embryo kidney, chick embryo fibroblasts, mouse fibroblasts, human amnion, and HeLa cells. In a note at the end of their paper, they added that "segmentation of the trophozoite" was subsequently observed in some of the cultures.

This report concerns the cultivation of a turkey coccidium, *E. meleagrimitis*, in monolayer cultures of bovine and porcine kidney cells.

### MATERIALS AND METHODS

OOCYSTS: Oocysts were obtained from the droppings of turkeys at the height of infection. The method for their recovery and sporulation was the same as that previously described (Doran and Farr, 1962). Only oocyst suspensions having more than 85% sporulated oocysts were used.

Bacteria-free oocyst suspensions were obtained by treatment with 5.25% sodium hypochlorite (undiluted Clorox) as described by Jackson (1964). They were stored in Ringer's solution at 3 to 6 C and were between 2 weeks and 3 months old when used.

EXCYSTATION OF SPOROZOITES: Sporocysts were released from oocysts by grinding with a mortar and pestle (Doran and Farr, 1962). They were concentrated by centrifugation at 1,500 *g* for 5 minutes and then treated for 15 to 18 minutes at 37 to 41 C with excystation solution [5% chicken or turkey bile in Ringer's solution + 0.25% trypsin (1–300, Nutritional Biochemicals Corp.)]. The solution, which had been Seitz filtered, quickly frozen, and stored in 5-ml aliquots at –40 C, was adjusted to pH 7.3 to 7.8 and used immediately after thawing. In several preliminary tests, between 85 and 95% of the available sporozoites excysted during the 15- to 18-minute interval.

CELL CULTURES: The source, origin, and maintenance media of the kidney cell cultures are listed in Table 1. All cultures were in Leighton tubes containing 10- by 22-mm cover glasses. They were kept at 41 C. Each experiment consisted of three to seven cultures for each time interval. Media also contained phenol red indicator (10 mg/ml), dihydrostreptomycin (100 μg/ml), penicillin (100 units/ml) and nystatin (100 units/ml). The maintenance media were changed when necessary to maintain the pH in each culture between 6.8 and 7.2.

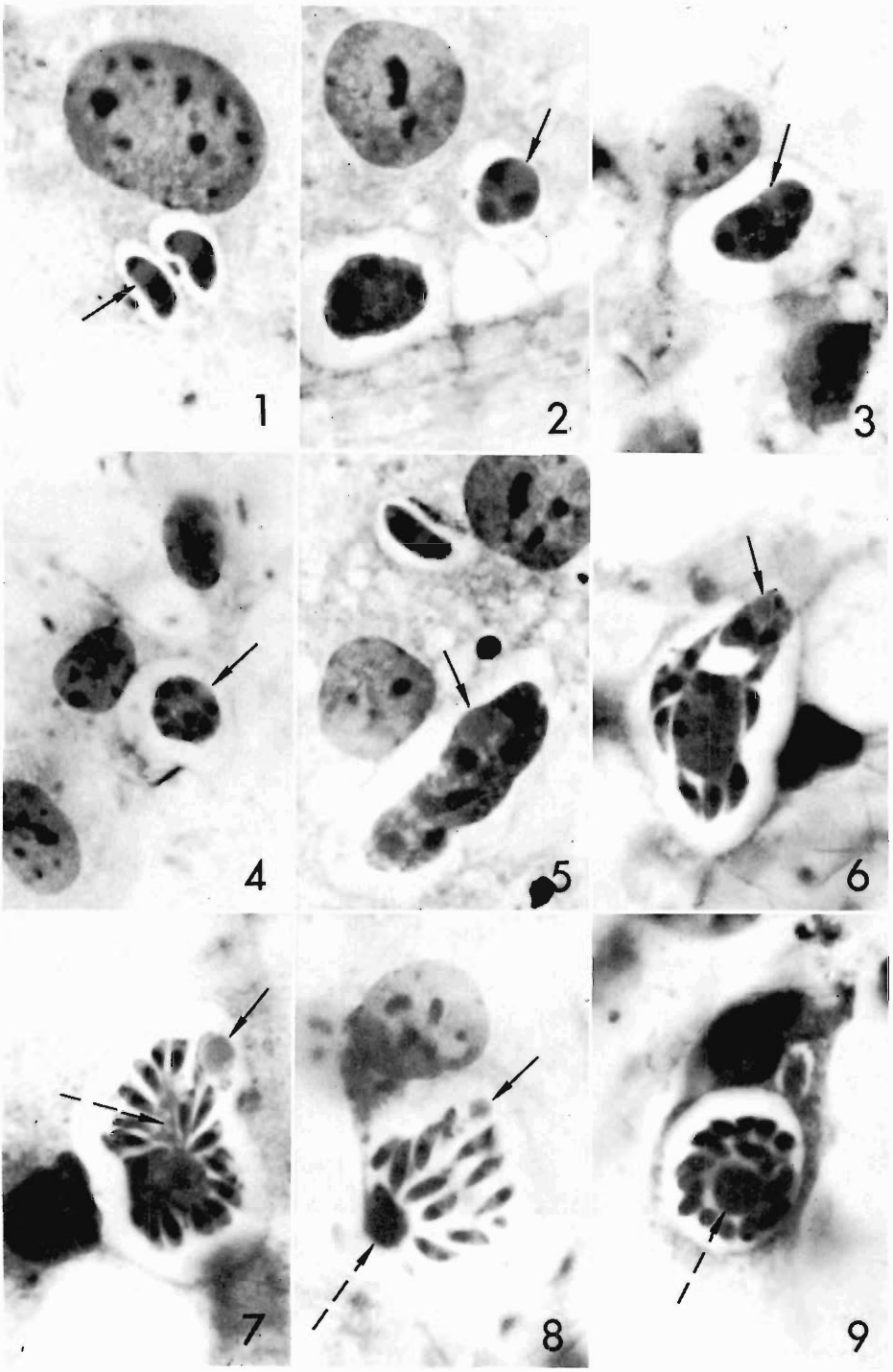


TABLE 1. Source, origin, and maintenance media of cell cultures.

| Cell type | Exper. number | Source | Origin | Serial passage number | Maintenance medium |
|---|---|---|---|---|---|
| Bovine (primary) | 1 | F | embryonic | | BME |
| | 2 | F | embryonic | | BME |
| | 3 | MA | embryonic | | BME |
| | 4 | MA | embryonic | | ELAC |
| Bovine (serial) | 1 | MA | embryonic | 10 | BME |
| | 2 | MA | embryonic | 17 | BME |
| | 3 | MA | embryonic | 20 | BME |
| Porcine (primary) | 1 | MA | embryonic | | BME |
| | 2 | MA | embryonic | | BME |
| | 3 | BPL | non-embryonic | | ELAC |
| Porcine (serial) | 1 | MA | non-embryonic | 12 | BME |
| | 2 | NADL | non-embryonic | 17 | ELAC |
| | 3 | BPL | non-embryonic | 13 | ELAC |
| | 4 | NADL | non-embryonic | 172 | ELAC |

F = Flow Laboratories, Rockville, Md.
MA = Microbiological Associates, Bethesda, Md.
BPL = Beltsville Parasitological Laboratory, prepared essentially by the method of Younger (1954).
NADL = National Animal Disease Laboratory, Ames, Iowa.
BME = Basal Medium Eagle (Eagle, 1955) with Earle's (Earle, 1943) balanced salt solution + 2% calf serum.
ELAC = Earle's B.S.S. (93%), lactalbumin hydrolysate (5%), and calf serum (2%).

INOCULATION OF CELL CULTURES: Excysted sporozoites were concentrated by centrifugation at 1,800 *g* for 5 minutes, washed once with the maintenance medium, and then resuspended in the volume necessary to infect the planned number of cultures. After the suspension had been quickly adjusted to pH 7.0 to 7.2, it was thoroughly agitated and 1.2 ml was pipetted into each cell culture. Only those cultures that were confluent and did not contain cell aggregates were used.

Inocula could not be accurately counted and calibrated because of differences in release of sporocysts, excystation of sporozoites, and especially the survival rate in the maintenance media. It was found (unpublished) that the sporozoites died quite rapidly in the absence of cells. Each experiment, however, was started with sufficient sporulated oocysts to yield theoretically about 15 million sporozoites per tube. Sporozoite-containing media were pipetted quickly into tubes to minimize fluctuations in number.

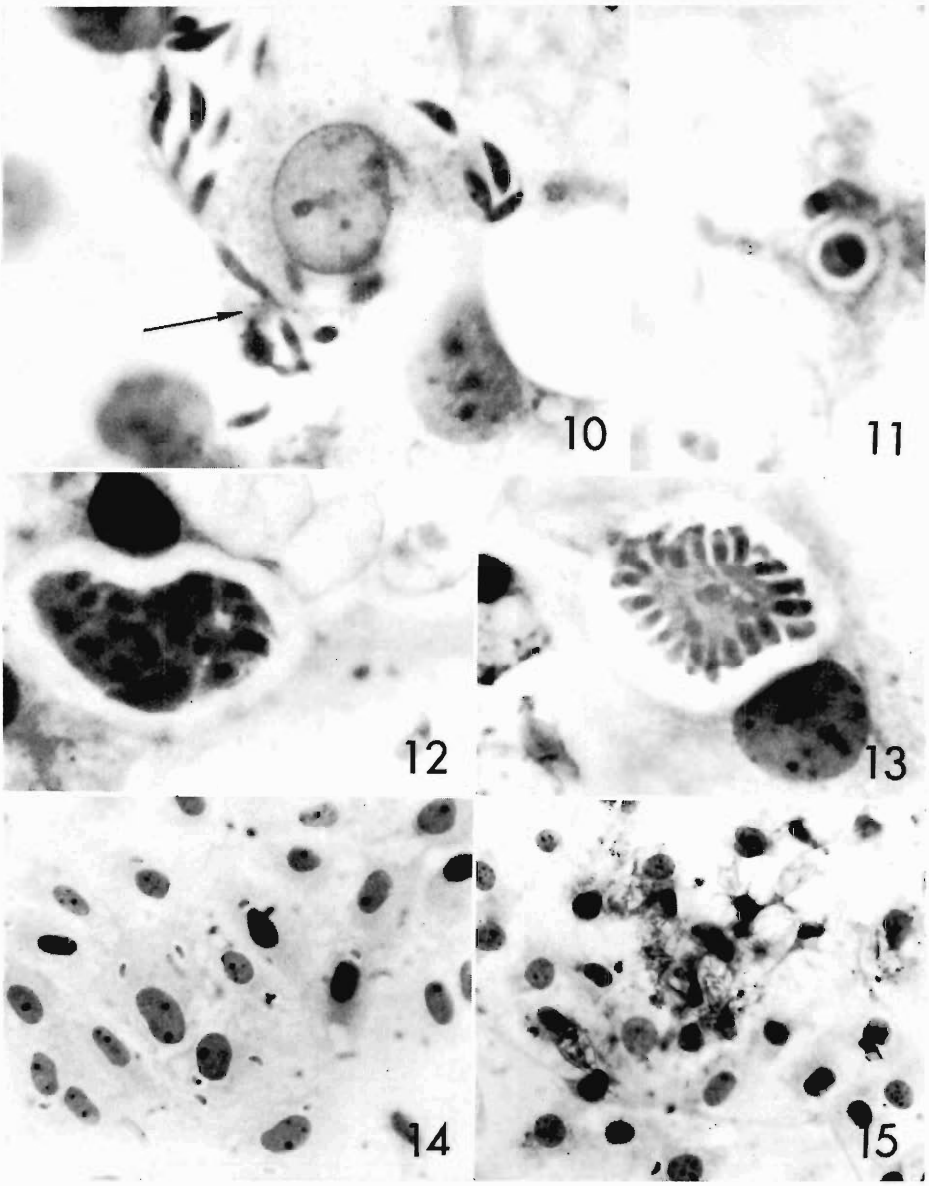
It was found in preliminary work that most of the sporozoites that entered cells did so by 2½ to 3 hours. Therefore, after 3 to 3½ hours at 41 C, the sporozoite-containing suspension was removed and the cells were washed twice with fresh maintenance medium. This removed some of the debris (unbroken oocysts, sporocysts, and oocyst and sporocyst hulls) from the cultures.

FIXING AND STAINING CELL CULTURES: At 24, 48, 72, 96, and 120 hours after inoculation, cover glasses were removed from the tubes, gently washed in warm phosphate buffered saline for about 3 seconds, and placed in aluminum wire baskets attached to glass slides. The cultures were then fixed in 10% neutral phosphate buffered formalin, stained with Harris' hematoxylin and eosin as recommended by Paul (1960), and mounted as permanent preparations on glass slides.

COUNTING: Counts were made using ×645 magnification. They represent those sporozoites and other developmental stages that

←

FIGS. 1–9. Magnification × 1,840. An eosinophilic globule is indicated by a solid arrow and a residual body by a broken arrow. 1. Two sporozoites within the cytoplasm of a cell. 5 hrs. 2. Two immature schizonts each containing three nuclei. Note that one of them does not have an eosinophilic globule. 72 hrs. 3. Immature schizont containing four nuclei. 48 hrs. 4. Immature schizont with eight nuclei. 48 hrs. 5. A sporozoite and a large, oblong, immature schizont with six nuclei. 72 hrs. 6. Immature schizont in which merozoite formation has commenced. The vacuole contains nine merozoites, some of which are still attached by their posterior ends. 72 hrs. 7. Schizont in which merozoite formation has been completed, but the merozoites are still attached to the rather large residual body. 48 hrs. 8. Mature schizont with detached merozoites and a smaller, rounded residual body. Note that the eosinophilic globule still persists. 9. Mature schizont, end view.




FIGS. 10–15. Magnification × 1,840, except Figure 11 (× 2,240) and Figures 14 and 15 (× 430). The eosinophilic globule is indicated by the arrow. 10. Merozoites of the first generation free and within an enlarged cell. 72 hrs. 11. Trophozoite, or "ring stage," of the second generation. A merozoite, partially out of focus, is immediately above the trophozoite. 72 hrs. 12. A large, immature schizont that does not contain an eosinophilic gobule. Note the sporocyst shells in upper right corner. 72 hrs. 13. Another immature schizont in which there is no eosinophilic globule. Merozoite formation has taken place, but they still remain attached by their posterior ends. 72 hrs. 14. Porcine cell culture (serial) showing the presence of sporozoites and the absence of debris in the inocula. 120 hrs. 15. Bovine cell culture (serial) showing the near absence of sporozoites and the presence of large amounts of debris in the inocula. 120 hrs.

TABLE 2. Number of sporozoites and schizonts found in bovine kidney (serial) cell cultures at 48, 72, and 96 hours after inoculation.

| Exper. no. | Culture no. | 48 hours | | | | | 72 hours | | | | | 96 hours | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sporozoites | Schizonts | | | | Sporozoites | Schizonts | | | | Sporozoites | Schizonts | | | |
| | | | with e.g. | | without e.g. | | | with e.g. | | without e.g. | | | with e.g. | | without e.g. | |
| | | | I | M | I | M | | I | M | I | M | | I | M | I | M |
| 1 | 1 | 1,414 | 48 | 13 | 0 | 0 | 607 | 27 | 16 | 5 | 0 | 380 | 5 | 2 | 5 | 0 |
| | 2 | 975 | 41 | 9 | 0 | 0 | 320 | 17 | 27 | 7 | 0 | 172 | 3 | 2 | 1 | 0 |
| | 3 | 1,920 | 29 | 27 | 0 | 0 | 870 | 14 | 14 | 9 | 0 | 312 | 9 | 1 | 1 | 0 |
| | totals | 4,309 | 118 | 49 | 0 | 0 | 1,797 | 58 | 57 | 21 | 0 | 864 | 17 | 4 | 7 | 0 |
| 2 | 1 | 1,112 | 39 | 1 | 0 | 0 | 970 | 19 | 19 | 3 | 0 | 240 | 9 | 0 | 2 | 0 |
| | 2 | 1,495 | 18 | 2 | 0 | 0 | 459 | 29 | 10 | 2 | 0 | 467 | 4 | 0 | 0 | 0 |
| | 3 | 2,000 | 27 | 4 | 0 | 0 | 1,002 | 22 | 12 | 0 | 0 | 325 | 0 | 1 | 4 | 0 |
| | totals | 4,607 | 84 | 7 | 0 | 0 | 2,431 | 70 | 41 | 5 | 0 | 1,032 | 13 | 1 | 6 | 0 |
| 3 | 1 | 1,320 | 27 | 2 | 0 | 0 | 820 | 9 | 9 | 14 | 0 | 175 | 3 | 4 | 2 | 0 |
| | 2 | 1,475 | 17 | 9 | 0 | 0 | 740 | 9 | 12 | 15 | 0 | 217 | 1 | 2 | 1 | 0 |
| | 3 | 1,505 | 30 | 15 | 0 | 0 | 683 | 7 | 4 | 10 | 0 | 321 | 3 | 2 | 3 | 0 |
| | totals | 4,300 | 74 | 26 | 0 | 0 | 2,243 | 25 | 25 | 39 | 0 | 713 | 7 | 8 | 6 | 0 |

Abbreviations: I = immature; M = mature; e.g. = eosinophilic globule.

were within cells on one-half of the cover glass. The area from which counts were made was always every other "row" across the length of the cover glass. The presence of a vacuole-like intracytoplasmic space around the parasite was used as criterion of intracellularity.

## RESULTS

Sporozoites entered cells in all of the primary and serial cultures of both bovine and porcine kidney. They were found in fibroblast-like cells as well as epithelial-like cells. Multiple infection of cells with sporozoites was common. When infection with four or less occurred, and all were less than one body length from one another, they were usually found side by side and oriented in the same direction (Fig. 1).

Schizogony occurred in only the epithelial-like cells in serial cultures of bovine kidney. Although many of the small number of epithelial-like cells present in some of the primary cultures contained sporozoites, schizonts were not observed.

Immature and mature schizonts of the first asexual generation (Figs. 2–9) were found at 48, 72, and 96 hours. Immature schizonts, in which merozoite formation had not yet begun (Figs. 2–5), measured 4 to 16 $\mu$ by 4 to 6 $\mu$. Mature schizonts containing 12 to 28 merozoites that were detached from a large residual body measured 13 to 18 $\mu$ by 12 to 14 $\mu$. The merozoites (Figs. 8, 10), each of which contained a prominent nucleus in the rounded posterior one-half of its body, measured 3.2 to 3.8 $\mu$ by 1.2 $\mu$. Trophozoites or "ring stages" (Fig. 11) and immature schizonts not having an eosinophilic ("refractile") globule (Figs. 2, 12, and 13) were found at 72 and 96 hours. The trophozoites were round and measured 1.5 to 2.0 $\mu$ in diameter. The immature schizonts were generally a few microns larger than those with a globule.

The number of schizonts with and without an eosinophilic globule found at each of the intervals was extremely small (Table 2). At 48 hours, less than 5% of the parasites in each culture were schizonts. At 72 hours, it was 3 to 14%, and at 96 hours, again less than 5%. At 120 hours, only a very small number (less than 100) of sporozoites were present.

Between 48 and 96 hours, there was a 69 to 89% decrease in the total number of parasites found in each of the cultures (Table 2). These losses were probably not because of a single factor. However, a major cause probably was the debris in the inocula. Washing three times with fresh maintenance medium was not equally effective in removing the oocyst and sporocyst hulls from the four types of cultures. Nearly all of the debris was removed from serial porcine cultures (Fig. 14), but very little was removed from the serial bovine cultures (Fig. 15). Sharma and Foster (1964) found that extracts of *E. tenella* oocysts were toxic to cells in rabbits. This is probably also true for cells grown *in vitro*. In the serial bovine cultures, cells died quickly after 48 hours and many of the dead cells contained degenerate parasites.

## DISCUSSION

An eosinophilic ("refractile") globule is considered characteristic of sporozoites and schizonts of only the first asexual generation of *E. meleagrimitis* (Clarkson, 1959; Tyzzer, 1929). The schizonts that lacked a globule were found 24 hours later than those with a globule and may well have been those of the second generation. However, it is not known, especially in cell culture, whether the eosinophilic globule regresses in size and disappears before merozoite formation in some schizonts but not in others.

Patton (1965) found mature first generation schizonts of *E. tenella* in bovine kidney cell cultures at 4 to 6 days with the greatest number at 5 days. In the chicken, 3 days were required for completion of the first generation (Tyzzer, 1929). The time intervals at which the early schizogonous stages of *E. meleagrimitis* were first found *in vitro* were surprisingly similar to those at which similar stages are found in the turkey. Clarkson (1959) reported immature and mature first generation schizonts at 24 and 48 hours, respectively. *In vitro*, no developmental stages were found at 24 hours, but both immature and mature schizonts were found at 48 hours. Clarkson also found trophozoites of the second generation at 48 hours and mature schizonts of the same generation at 66 hours. *In vitro*, trophozoites also occurred at 48 hours and immature schizonts, if actually those of the second generation, were seen at 72 hours. Although the time intervals for appearance of developmental stages *in vivo* and *in vitro* were similar, morphology of these stages was quite different. Clarkson reported 80 to 100 merozoites measuring 4.5 by 1.5 $\mu$ within a first generation schizont. *In vitro*, schizonts contained only 18 to 28 merozoites that measured 2.9 to 3.5 $\mu$ by 1.4 $\mu$. Clarkson also found second generation mature schizonts measuring only 8 by 7 $\mu$ and containing 8 to 16 merozoites. *In vitro*, the immature schizonts without an eosinophilic globule were nearly twice as large and many of them (Fig. 13) already contained more than 16 merozoites. Cell culture, especially monolayer, is a highly artificial system. One should expect some variation. Even so, it will be interesting to compare the present morphological findings with those of the same species grown in monolayer cultures of the definitive host cell.

It was rather surprising that no development was observed in serial porcine cells. The epithelial-like cells were in excellent condition and many sporozoites were present after 120 hours. The serial porcine cells, as well as the primary porcine and bovine cells which were also in good condition and contained sporozoites, apparently lacked the necessary intrinsic cell stimulus to initiate development. Cessation of development in the serial bovine cells was probably because of inadequate cell stimulus. The stimulus for further development may (1) never have been inherently present in the cells or (2) diminished or lost because of inadequate growth conditions imposed on the cultures by the debris in the inocula.

## SUMMARY

*Eimeria meleagrimitis*, a parasite of the duodenal epithelium of turkeys, completed one asexual generation and perhaps part of another in monolayer serial cultures of bovine kidney cells. Development did not take place in primary bovine, primary porcine, or serial porcine cell cultures.

Although the time intervals at which the early schizogonous stages were first found *in vitro* were similar to those at which similar stages were found in the turkey, the morphology was quite different. Mature first generation schizonts contained only 18 to 28 merozoites. Immature schizonts, which lacked an eosinophilic ("refractile") globule and might have been second generation, were nearly twice the size of those in the host.

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# Intestinal Protozoans and Parasites of the Gelada Baboon (*Theropithecus gelada* Rüppel, 1835)*

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Even though baboons range throughout the greater part of Africa, the monotypic *Theropithecus gelada* occurs only in a restricted area associated with the highlands of Ethiopia. The gelada, essentially a terrestrial species, occurs in numbers in its habitat and has been known to man for many decades. However, the seclusion of undeveloped areas of Ethiopia and the exportation of limited numbers of animals seems to account for the rarity of this primate in zoos and zoological gardens. A number of protozoans and helminth parasites have been reported for other species of baboons (Myers and Kuntz, 1965), but there are only a few records for the gelada.

The present report, based upon an examination of a short series of animals in San Antonio Zoo, provides additional information to our limited parasitological knowledge of *T. gelada*.

## MATERIALS AND METHODS

Thirteen gelada baboons, obtained through an animal importer, were examined shortly after arrival and before introduction into the San Antonio Zoo. Data and information were obtained by an examination of stools from 13 hosts, plus postmortem examination of two others. Fecal samples were preserved by the MIF (merthiolate-iodine-formalin) vial technic (Sapero and Lawless, 1953). The incidence for intestinal protozoa and helminths is based upon a combination of the direct smear from sediments in the vial and the MIFC concentration technic (Blagg, et al., 1955). A search for blood parasites was based on an examination of two thin smears stained in Giemsa. Standard parasitological procedures were employed for complete postmortem examinations. All tissues were macerated and washed in several changes of water to allow efficient removal of parasites. Nematodes were cleared in phenol to facilitate identifications.

## RESULTS AND DISCUSSIONS

The incidence of intestinal protozoans and helminths determined by examination of a single fecal sample from 13 hosts, plus two autopsy recordings, is presented in Table 1. *Oesophagostomum bifurcum*, *Trichostrongylus colubriformis* and a pentastome nymph (*Linguatula serrata*) were obtained at the time of postmortem examinations. Although the listing is not extensive, it represents the largest sampling made at one time for the gelada baboons.

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TABLE 1. Intestinal Protozoans and Helminths of *Theropithecus gelada*.

| | |
|---|---|
| **PROTOZOA** | |
| *Endolimax nana* (Wenyon and O'Connor, 1917) Brug, 1918 | 3/13 |
| *Entamoeba coli* (Grassi, 1879) Casagrandi and Bargagallo, 1895 | 5/13 |
| *Entamoeba histolytica* Schaudinn, 1903 | 1/13 |
| *Iodamoeba bütschlii* (von Prowazek, 1911) Dobell, 1911 | 1/13 |
| **HELMINTHS** | |
| *Heterodera* sp. | 1/13 |
| *Oesophagostomum bifurcum* (Creplin, 1894) Railliet and Henry, 1906* | 1/13 |
| *Trichostrongylus colubriformis* (Giles, 1892) Ransom, 1911* | 2/13 |
| *Trichuris* sp. | 2/13 |
| **ARTHROPODS** | |
| *Linguatula serrata* Frölich, 1789 (nymph)* | 1/13 |

* Identification based upon specimens obtained at autopsy.

Most of the few reports on the parasites of *Theropithecus* have been concerned with helminths, and particularly cestode cysts, removed at postmortem examination of the animals maintained in zoos and zoological gardens (Myers and Kuntz, 1965). Apparently, only two protozoans, *Entamoeba histolytica* and *Entamoeba chattoni* (Salis, 1941) and two intestinal helminths, *Physaloptera turgida* (Canavan, 1929) and *Trichurus* (Fiennes, 1966) have been previously reported from this host. While not unexpected in baboons, the other protozoans and helminths listed in Table 1 are considered as new host records.

An examination of anesthetized animals revealed that these primates were much cleaner than the many Kenya baboons, *Papio doguera* Pucheran, 1856, which we have processed previously (Kuntz and Myers, in press), and there were no ectoparasites. No parasites were detected in vaginal samples or in peripheral blood smears. In contrast with the conditions generally present in *P. doguera* (Kuntz and Myers, unpublished data), oral examinations of the geladas revealed unusually clean mouths, with the absence of *Trichomonas* and *Entamoeba*. The latter commensals frequently are associated with the accumulation of tartar, organic detritus and lesions in captive *P. doguera*.

## SUMMARY

An examination of fecal samples from 13 *Theropithecus gelada*, plus postmortem findings of 2 hosts, revealed 1 parasitic (*Entamoeba histolytica*) and 3 commensal protozoans, as well as 4 species of nematodes and a larval pentastome. *Endolimax nana*, *Entamoeba coli*, *Iodamoeba bütschlii*, *Oesophagostomum bifurcum*, *Trichostrongylus colubriformis*, and *Linguatula serrata* are new records for the gelada baboon.

## ACKNOWLEDGMENTS

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# *Monordotaenia* nom. nov. for the Badger Taeniid Cestodes with One Row of Hooks

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Rausch (1947) pointed out the uniqueness of a single row of hooks in the genus *Taenia* in his redescription of *Taenia taxidiensis* Skinker, 1935, from the badger, *Taxidea taxus taxus*. This redescription was in order since the original paper on *T. taxidiensis* apparently was based on one complete specimen and several fragments. Skinker (1935), not knowing that the worm normally contained only a single row of hooks, erroneously assumed that the "other row" had been lost and placed the cestode in the genus *Taenia*.

Later, Honess (1937) found four specimens of taeniid containing a single row of hooks, also from the badger, which he described and named *Fossor angertrudae*. Wardle and McLeod (1952) referred to *F. angertrudae* as a "small form" since its length appeared as 39 to 46 mm in the original description. The measurements should have probably read 39 to 46 cm since the holotype, USNM No. 9053 is 382 mm in length. However, these latter authors suggested that *F. angertrudae* be regarded as "sub judice" until further records were established.

Skinker's holotype apparently has been lost and due to a mixup was not assigned 39803, the number in her publication. It now becomes necessary to designate the paratype slide USNM No. 32840 as the lectotype, although this specimen is not complete. The paratype USNM No. 32840 #1, marked hooks, is defective as no hooks are present. Therefore, Table 1 is based on Rausch's redescription of *T. taxidiensis* and measurements of the holotype of *F. angertrudae*, Honess, 1937, USNM No. 9053, by the present writer. The measurements of the two worms, except the scoleces and suckers, are strikingly similar. The exceptions are no doubt due to techniques in preparation as the scolex and suckers in Skinker's paratype USNM No. 32840 measured 740 and 200 mm, respectively.

Upon reviewing *Fossor angertrudae* preserved on three slides marked USNM No. 9053, the paratype from the Wyoming Agricultural Experimental Station and the paratype of *Taenia taxidiensis* on six slides marked 32840, I have concluded that *F. angertrudae* is synonymous with *T. taxidiensis*.

The genus *Fossor*, containing the single member *F. angertrudae*, was created to separate the species from *Taenia*, a genus in which all members possess a double crown of hooks. It has already been pointed out that Skinker did not realize she was working with material that contained only one row of hooks and therefore she naturally placed the worm in the genus *Taenia*. However, since Honess' generic name, *Fossor*, is a junior homonym of *Fossor* Lichtenstein, 1844, which is presently a synonym of the rodent genus *Georychus* Illiger 1833, *Monordotaenia* nom. nov. is proposed here for the badger cestode containing a single row of hooks. The correct name becomes *Monordotaenia taxidiensis*, Skinker, 1935, new combination.

## DISCUSSION

The major reason why this badger worm has been confused is that the paucity of Skinker's

TABLE 1. Comparison of *Taenia taxidiensis* and *Fossor angertrudae*.

| Worm Source | *T. taxidiensis* Rausch, 1947 | *F. angertrudae* No. USNM 9053 |
|---|---|---|
| Strobila (mm) | 480.0 | 382.0 |
| Width (mm) | 3.0 | 2.6 |
| Segments gravid (length mm) | 8.0–8.5 | 7.3 |
| Scolex ($\mu$) | 570.0 | 780.0 |
| Suckers ($\mu$) | 156.0 | 200.0 |
| No. of hooks | 20–27 | 20 |
| Size of hooks ($\mu$) | 90.0 | 89.0 |
| Hook guards ($\mu$) | 46.0 | 43.0 |
| Ventral canals ($\mu$) | 142.0 | 140.0 |
| Dorsal canals ($\mu$) | 20.0 | 18.0 |
| Cirrus length ($\mu$) | 240.0–330.0 | 270.0 |
| Cirrus width ($\mu$) | 100.0 | 110.0 |
| No. of testes | 200–300 | 200–300 |
| Size of testes ($\mu$) | 70.0–90.0 | 60.0–80.0 |
| Host | *Taxidea taxus* (Schreber) | *Taxidea taxus* (Schreber) |
| Locality | Wisconsin | Wyoming |

material prevented her from making a correct assessment in the original publication. Wardle and McLeod mentioned that Skinker's badger cestode contained one row of hooks, but it was nevertheless placed in *Taenia*, i.e., with worms containing two circles of hooks. It is surprising that Rausch, in his redescription, did not remove this badger worm from the genus *Taenia*.

It would seem unlikely that the other taeniid with a single row of hooks, *Taenia monostephanos* Linstow 1905, reported in the lynx from Russia, belongs to the genus *Taenia*. However, until more information is accessible, this worm must be regarded as a *species inquirenda*.

ACKNOWLEDGMENTS

I wish to thank Dr. W. W. Becklund of the Beltsville Parasitological Laboratory and Dr. R. F. Honess, University of Wyoming, who kindly provided me with the material employed in this study.

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## Feeding of *Xiphinema index* and *X. diversicaudatum*

J. M. FISHER* AND D. J. RASKI**

Information on feeding and feeding sites of *Xiphinema* spp., vectors of plant viruses, would be useful in studying acquisition and transmission of the viruses by the various stages of the nematodes. Indirect observations (Pitcher and Posnette, 1963) and pathological symptoms (Davis and Jenkins, 1960; Schindler and Braun, 1957; Schindler, 1957; Raski and Radewald, 1958; Radewald, 1962) indicate some of the feeding sites of *Xiphinema index* and *Xiphinema diversicaudatum* on certain hosts. Feeding of *X. index* on roots of grape growing on 2% agar was observed directly (Radewald, 1962; Radewald and Raski, 1962) but complete and detailed description of the activities are lacking. Pitcher and Posnette (1963) showed that the onchiostyle of a single specimen of *X. diversicaudatum* could penetrate as far as the stele of small roots (< 0.25 mm diam.), but further observations are necessary to substantiate that *Xiphinema* spp. are regularly capable of such deep feeding in roots.

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### METHODS

Feeding of *X. index* was observed on seedling grapes, *Vitis vinifera* L., var. Mission, growing in 0.75% distilled water-agar culture in petri dishes. The initial agar medium prepared with copper-distilled water was lethal to the nematodes, killing them in six hours. The effect was due presumably to copper ions (van Gundy and Thomason, 1962) and glass-distilled water was used thereafter without difficulty. Several agar concentrations were tried, with little effect on the nematodes, but because of the high evaporation rate at Davis, it was found that a low agar concentration (0.75% Difco

agar) allowed the culture medium to be kept longer in a condition suitable for the growth of the nematodes and the seedlings. No attempt was made to work under aseptic conditions. To reduce the problem of microorganisms, no nutrients were added to the agar medium. Germinating grape seedlings can grow for three to four weeks on the reserve food of the cotyledons. Gall formation on roots, egg-laying, embryological development, moulting and feeding all occurred in one or other of the cultures. All cultures were kept on the laboratory bench at 20 to 22 C.

## OBSERVATIONS

The total time for embryological development to hatching was 6 to 8 days. No moult was observed in the egg which agrees with the observations of Radewald (1962) who found the first-stage larvae moulted 24–48 hours after emergence. Mature larvae within the egg had a second onchiostyle inserted within the extension of the first stylet in such a way that the tip of the second onchiostyle almost reached to the base of the first odontostyle. On hatching the presence of the second onchiostyle did not prevent feeding.

Precise measurements were not taken which would permit exact identification of hatched larvae as to first- or second-stage. Consequently these are referred to as newly-hatched larvae. These newly-hatched larvae fed well back from the root tip on the outer cortical cells of the piliferous region (Fig. 1). The onchiostyles of these larvae were not long enough to penetrate deeper into the root and in this region no symptoms were produced. Shortly before moulting they migrated to the root tip where they fed for a short time, and small galls were produced. After this short feeding period these larvae migrated into the medium in advance of the root tip and moulted.

The same two areas of the root were fed on by the next larval stage but these larvae showed greater ambivalence regarding feeding site preference, migrating back and forth from piliferous region to root tip. Again no symptoms were produced by feeding in the piliferous region, but galls were formed as a result of feeding at the root tip.

The time spent at one feeding site was quite variable and in some cases switching on of the

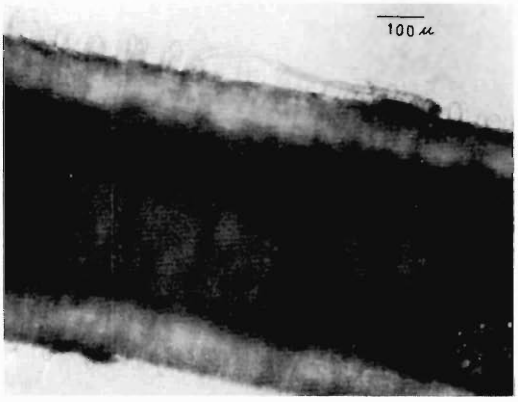


Fig. 1. Young larval stage of *X. index* feeding in the piliferous region of a grape root.

microscope light or movement of the petri dish disturbed some of the feeding nematodes. After a short time, even in the light, most of the nematodes resumed feeding, either at the same or a different site. The older larval stages and adult females (no males were present in the population used) always fed at the root tip, mainly slightly in advance of, or slightly behind, the meristematic cells (Fig. 2). The onchiostyle penetrated to the meristem either through the root cap cells or through that part of the root epidermis just behind the root cap, where the diameter of a grape root narrows considerably. Pauses in the progress of the onchiostyle while penetrating outer cortical cells, accompanied by muscular contractions of the oesophageal bulb appeared to indicate injection or withdrawal of material to or from the root cells. No material was ever observed to pass along the lumen of the oesophagus in either direction but contractions of the oesophageal bulb frequently were seen. Often in penetration, the stylet would cease moving and muscular contraction of the posterior part of the oesophagus would take place for a variable length of time up to 2 minutes in each successive cell as the onchiostyle was pushed deeper into the rootlet. The onchiostyle was not limited to straight penetration but had enough lateral movement to allow penetration of adjacent cells in the same optical plane. Bending of the onchiostyle as shown by Pitcher and Posnette (1963), while not common, was seen on a number of occasions. Penetration of

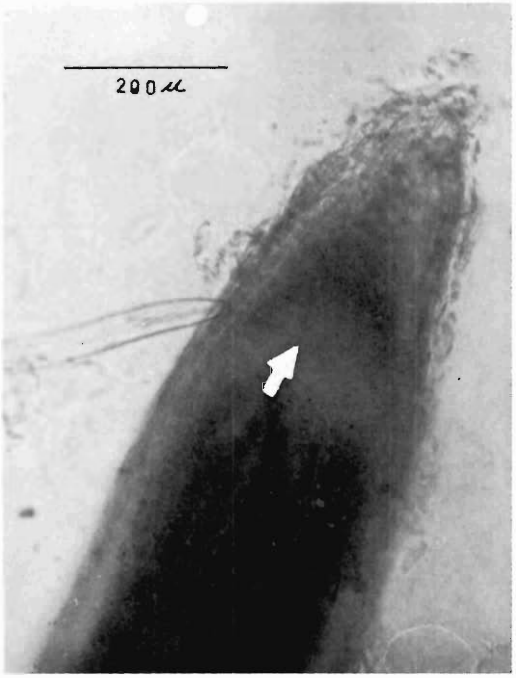


Fig. 2. *X. index* feeding in the meristematic region of a grape root tip.

two adjacent cells involved slight withdrawal of the onchiostyle, lateral movement and further penetration.

The onchiostyle often penetrated very deeply but in no case were stylet extensions exserted. Exsertion of the stylet reached its maximum when its base was at the level of the base of the lips; thus the length of onchiostyle penetrating the root depended on the age of the nematode. The maximum depth of penetration also depended on the diameter of the root. For feeding to take place, it was not necessary for the onchiostyle to be fully exserted.

An effect on the walls of epidermal cells of the root, following passage of the onchiostyle was noticed. Initial penetration by the onchiostyle appeared to be mainly intercellular. The epidermal cell walls became markedly outlined (Fig. 3) indicating some change in structure, perhaps even slight separation of the cells. This effect was observed only at root tips.

Some large galls were formed on the root tips (Fig. 4). Where a number of nematodes had fed on different sides of the same root

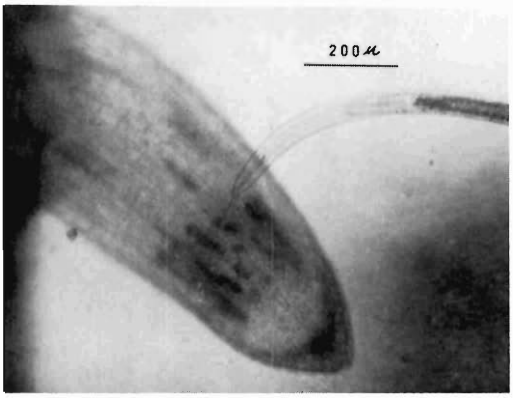


Fig. 3. Demarcation of the epidermal cell walls of a grape root as a result of nematode feeding.

tip, elongation of the root was prevented, resulting in a swollen root tip with a reduced number of root cap cells and a truncated appearance. When feeding took place on only one side of the root tip, galling was restricted to one side. The shortest time for visible gall formation was 24 hours from the time of inoculation with the nematodes. Large galls, which are common in pot cultures and in the field, did not develop in petri dish cultures. In all cultures, nematodes ceased feeding as soon as the roots stopped growing.

The foregoing descriptions refer to the feeding of *X. index* and the work was done at Davis, California. Similar observations have been made recently with *X. diversicaudatum* in Adelaide. Attempts to feed the nematodes on some herbaceous hosts of viruses (cucumber, petunia and white clover) failed, but the nematodes did feed and produce galls on rose roots. *X. diversicaudatum* was invariably more sensitive to disturbance than *X. index*, but feeding sometimes recommenced a short time after exposure to a new set of conditions.

Although feeding at each site was quite variable, one example is given in detail. Once the lips of the nematode made suitable contact with the root the onchiostyle penetrated the epidermal cell almost immediately. This was followed by intermittent contractions of the basal region of the oesophagus of approximately 2-second duration; between contractions were periods of inactivity. This lasted for 5 minutes at the end of which time continuous

contractions of the bulb lasted for 2 minutes. When the bulb stopped its action, the onchiostyle was pushed deeper into the second layer of cells. The intermittent contractions of the oesophageal bulb then lasted for approximately 1 minute and this was immediately followed by successive but gradually lengthening periods of pulsation of the bulb. The onchiostyle remained in this cell for 3 minutes and then penetrated the next layer of cells. This procedure was repeated as the onchiostyle was pushed deeper into the root. When contact with the root was broken, the lips often lost contact with the root surface before the onchiostyle was fully withdrawn, so that the tip of the onchiostyle was washed by the fluid of the medium.

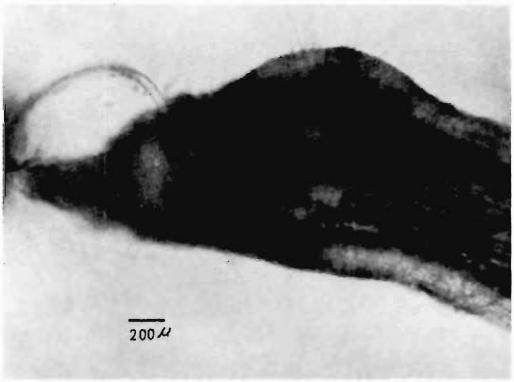


Fig. 4. Galling of grape root tips in petri dish culture.

## DISCUSSION

Although many observations were possible, the disadvantages of the limited period of growth of the host were considerable. In following the life cycle of *X. index* it was necessary to remove the nematodes to a new seedling every three to four weeks. Once the onchiostyle had penetrated deeply, grape roots were too thick for viewing the actual cell in which feeding was taking place. Perhaps another host would be more suitable.

The problem of relating the habits of nematodes in culture to those in soil still exists. Thus the large galls which are observed in pot cultures and in the field were not seen in culture. This could have been due to a more transitory type of feeding in culture, smaller numbers of nematodes, or the different conditions and nutrient status for growth of roots.

Symptoms were produced when young larvae fed at root-tips but not when the same larvae fed in the piliferous region of the root. This suggests that immature, undifferentiated root cells are necessary for symptom production. Feeding in these different sites may also affect the efficiency of different stages to acquire and transmit viruses. During experiments on transmission of viruses to herbaceous hosts symptoms usually are not produced by the nematodes. While this may be due to feeding only in the piliferous regions of these hosts the failure to observe feeding at any site on herbaceous hosts in these experiments suggests that there may be a different feeding process, or that the host–parasite relation on these plants may be different.

## SUMMARY

Feeding of *Xiphinema index* and *X. diversicaudatum* on roots of germinating seedlings in petri dish cultures is described. Young larvae of *X. index* fed in the piliferous region as well as at the root tip but galls were produced only at the tips. The presence of a second onchiostyle within the stylet extension of these larvae did not prevent feeding. Females fed only at the root tip and galls were evident within 24 hrs after inoculation. Maximum observed exsertion of the stylet was when the base of the onchiostyle was level with the base of the lips and thus maximum penetration of the root depended on the length of the stylet. *X. diversicaudatum* was more sensitive to disturbance while feeding than *X. index*.

## ACKNOWLEDGMENTS

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## On the Classification of the Insect Parasitic Nematodes of the Sphaerulariidae Lubbock, 1861 (Tylenchoidea: Nematoda)

W. R. NICKLE[1]

One of the first obligate tylenchid parasites of insects was described by Dufour in 1837 as *Sphaerularia bombi* from the bumblebees, *Bombus terrestris* and *Bombus hortorus*. In 1861, Lubbock proposed the family Sphaerulariaceae to contain the genus *Sphaerularia*. Other significant early contributions to the taxonomy of these forms include: Leuckart (1884), von Linstow (1890), zur Strassen (1892), Cobb (1920, 1921), Micoletzky (1922), T. Goodey (1930), Filipjev (1934), Thorne (1935), Fuchs (1915, 1929, 1933, 1938), Bovien (1937), Currie (1937), Chitwood and Chitwood (1937), Christie (1938) and Schneider (1939). More recent contributions include: T. Goodey (1953), Wachek (1955), Rühm (1956), Massey (1956, 1957, 1958, 1960, 1962), J. B. Goodey (1956, 1963), Khan (1957a, 1957b, 1960), Skarbilovich (1947, 1959), Welch (1959), and Nickle (1963a, 1963b). Fuchs, Wachek, and Rühm each published comprehensive works, and did much to clarify the systematics of this family. Bovien's publications on insect nematodes were outstanding and are classics today.

At present, 4 subfamilies, 21 genera, and 116 species are included in the Sphaerulariidae. Generally the classification is strong at the generic level, but at the specific level it is rather weak because detailed descriptions of free-living males and females are lacking. A tylenchid species can generally parasitize hosts in one genus, and unlike the mermithids, adults are found in adult hosts. Therefore, knowledge of the host aids in identification.

In several of the nematode genera the dorsal esophageal gland orifice is located at least one stylet length behind the base of the stylet or could not be found. The subventral esophageal glands may empty into the lumen of the esophagus a short distance behind the dorsal gland orifice. This led Wachek (1955) to place *Sphaerularia*, *Tripius*, and *Scatonema* in the Aphelenchoidea, though they lack the typical

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aphelenchoid median bulb and in all other respects appear to be tylenchoid.

Recently, I observed the dorsal esophageal gland orifice in the fourth stage female larva of *Sphaerularia bombi* and found that it was located behind the stylet in the normal tylenchoid position. Further, the presence of tylenchoid spicules and a gubernaculum in these forms also, supports their tylenchoid status. For these reasons I question the aphelenchoid status of these genera, and I now consider some of the genera placed in the Aphelenchoidea as belonging to the Tylenchoidea.

The problem inherent in making this change is that Sphaerulariaceae Lubbock, 1861 is an older family name than Allantonematidae (Pereira, 1931) Chitwood and Chitwood, 1937. In my opinion the insect parasitic Tylenchoidea merit only family rank along with the plant parasitic families; Tylenchidae, Heteroderidae, Criconematidae, Neotylenchidae, Hoplolaimidae, and Tylenchulidae. Future work in the group may necessitate the creation of a superfamily but at present, all insect-parasitic Tylenchoidea should be placed in the family Sphaerulariidae Lubbock, 1861; the Allantonematidae (Pereira, 1931) Chitwood and Chitwood, 1937 should be given the rank of subfamily.

*Entaphelenchus* Wachek, 1955 and *Peraphelenchus* Wachek, 1955 are endoparasitic tylenchids, having the typical aphelenchoid median bulb and spicules which are shaped like rose thorns. The gubernaculum is lacking, and in the writer's opinion, only these two genera are typical of the Aphelenchoidea.

During the last 8 years, I have studied representative specimens from 15 of the 22 genera belonging to the families Sphaerulariidae and Allantonematidae before making the revisions contained herein. It is hoped that this compilation will stimulate work in this interesting but neglected group.

Economically, insect parasitic nematodes of the Sphaerulariidae annually reduce insect populations by untold millions, and are indeed important self-perpetuating biological control agents. However, little effort has been made in manipulating, or exploiting these parasites for biological control, but the time is ripe for this type of endeavor.

Because of the brevity of the original description, the family diagnosis is emended. Brief diagnoses of the sub-families and genera are given and a new genus is established. Synonymies are presented along with my comments on the status of controversial groups.

The characters that typify the family include: absence of a valved median bulb; unique ovary of the free-living female; enlargement of female nematode in the body cavity of host, resulting in the nematode becoming a reproductive sac (heteromorphism), or female nematode with a prolapsed swollen uterus; obligate insect or mite parasitism.

FAMILY:

Sphaerulariidae Lubbock, 1861.

Syn. Sphaerulariaceae Lubbock, 1861.

DIAGNOSIS (Emended): Tylenchoidea. Usually with three distinct forms. Two free-living, slender, 0.5–1.0 mm long, often found in habitat of young host and one adult parasitic form found in haemocoel of insect or mite. (1) FL♀*: Stylet or pseudostylet rarely absent. Esophagus without median valvular bulb; ampullae near gland orifices often strongly developed; dorsal gland orifice not always found immediately behind spear, may be midway between spear and subventral gland orifices; esophageal glands long, overlap intestine. Gonad diagnostic; prodelphic; ovary small, finger-like with few oocytes; oviduct short; uterus prominent, temporarily packed with individual sperm after copulation. Becomes infective stage. Eggs absent. (2) FL♂: Usually slightly longer than female, not infective. Stylet may be absent. Esophagus weakly developed. Spicules usually tylenchoid, may be elaborate. Gubernaculum usually present. Caudal alae often present and peloderan. (3) AP♀: Obligate parasite in haemocoel of insects or mites. Large swollen reproductive sac, produced by expansion of free-living female; or, with uterus prolapsed, swollen. Stylet present or rarely absent, may be retracted. Esophagus and intestine usually degenerate at expense of reproductive system which often fills 80% of body. Oviparous or ovoviviparous; ovary usually flexed one or more times; rachis common.

* FL♀: Free-living female. FL♂: Free-living male. AP♀: Body cavity adult parasitic female.

PROPOSED CLASSIFICATION OF THE SPHAERULARIIDAE

Sphaerulariidae Lubbock, 1861.
Syn. Sphaerulariaceae Lubbock, 1861.
Sphaerulariinae (Lubbock, 1861) Pereira, 1931.
*Sphaerularia* Dufour, 1837.
**Sphaerulariopsis* Wachek, 1955.
**Tripius* Chitwood, 1935.
Syn. *Asconema* Leuckart, 1886.
*Atractonema* (Leuckart, 1886) Leuckart, 1887.
*Proatractonema* Bovien, 1944.
Allantonematinae Pereira, 1931. (Emended Chitwood, 1935).
Syn. Allantoneminae Pereira, 1931.
Allantonematidae (Pereira, 1931) Chitwood and Chitwood, 1937.
Contortylenchidae Rühm, 1956.
*Allantonema* Leuckart, 1884.
Syn. *Tylenchomorphus* Fuchs, 1915.
**Aphelenchulus* Cobb, 1920.
**Bovienema* Nickle, 1963.
**Bradynema* zur Strassen, 1892.
**Chondronema* Christie and Chitwood, 1931.
**Contortylenchus* Rühm, 1956.
**Heterotylenchus* Bovien, 1937.
**Howardula* Cobb, 1921.
Syn. *Tylenchinema* T. Goodey, 1930.
**Prothallonema* Christie, 1938.
*Metaparasitylenchus* Wachek, 1955. n. grad.
**Neoparasitylenchus* n. gen.
*Parasitylenchoides* Wachek, 1955.
**Parasitylenchus* Micoletzky, 1922.
Syn. *Polymorphotylenchus* Rühm, 1956.
*Proparasitylenchus* Wachek, 1955. n. grad.
*Protylenchus* Wachek, 1955.
**Sulphuretylenchus* Rühm, 1956. n. grad.
*Scatonema* Bovien, 1932.
*Dotylaphus* Andrassy, 1958. (genus inquirenda).
Fergusobiinae J. B. Goodey, 1963.
Syn. Fergusobiidae Siddiqi and J. B. Goodey, 1963.
**Fergusobia* Currie, 1937.
Syn. *Anguillulina* (*Fergusobia*) Currie, 1937.
Iotonchiinae T. Goodey, 1953.
Syn. Iotonchiidae (T. Goodey, 1953) Skarbilovich, 1959.
**Iotonchium* Cobb, 1920.

* Specimens of these genera were studied for this review.

SUBFAMILY:

Sphaerulariinae (Lubbock, 1861) Pereira, 1931.

DIAGNOSIS (Emended): Sphaerulariidae. FL♀: Stylet present, with or without knobs. Without eggs in uterus. FL♂: With tylenchoid anterior end. Stylet present, with or without knobs. Caudal alae present or absent. Spicules and gubernaculum tylenchoid. AP♀: Uterus everted partially or completely.

Genus *Sphaerularia* Dufour, 1837
(Figs. 2Z, 3V, 5A)

FL♀: Stylet present, without basal knobs. Dorsal gland orifice opens just behind stylet. FL♂: Stylet present, not prominent, without knobs. Caudal alae absent. Spicules and gubernaculum tylenchoid. AP♀: Uterus completely everted, greatly enlarged, larger and not as smooth as *Sphaerulariopsis*, becomes 15,000–20,000 times the volume of original female body which remains as a minute appendage to uterus, though often wrinkled and deformed. Oviparous.

HOST INSECTS:

Hymenoptera; *Bombus*, *Vespa*, *Psithyrus*.

TYPE SPECIES:

*Sphaerularia bombi* Dufour, 1837.

Syn. *Tylenchus bombi* (Dufour, 1837) Cobb, 1890.

Genus: *Sphaerulariopsis* Wachek, 1955
(Figs. 2W, 3W, 5A)

FL♀: Stylet present, well-developed, with basal knobs which may be irregular in size. Dorsal gland orifice opens anteriorly behind stylet. FL♂: Stylet present, with knobs. Caudal alae peloderan. Spicules and gubernaculum tylenchoid. AP♀: Uterus completely everted, greatly enlarged, smoother and not as large as *Sphaerularia*, original female body wrinkled and deformed, remains as an appendage to uterus. Oviparous.

HOST INSECTS:

Coleoptera; *Ernobius*, *Pissodes*, *Dendroctonus*. Hymenoptera; *Coeloides*.

TYPE SPECIES:

*Sphaerulariopsis stammeri* Wachek, 1955.

Syn. *Stictylus stammeri* (Wachek, 1955) Rühm, 1956.

OTHER SPECIES:

S. *dendroctoni* (Massey, 1956) Nickle, 1963.
Syn. *Sphaerularia dendroctoni* Massey, 1956.

S. *hastatus* (Khan, 1957) Nickle, 1963.
Syn. *Sphaerularia hastata* Khan, 1957.
*Stictylus hastatus* (Khan, 1957) Khan, 1960.

S. *pini* (Fuchs, 1929) Nickle, 1963.
Syn. *Tylenchus sulphureus pini* Fuchs, 1929.
*Allantonema sulphureus pini* (Fuchs, 1929) Filipjev, 1934.
*Parasitylenchus sulphureus* f. *pini* (Fuchs, 1929) Schneider, 1939.
*Allantonema pini* (Fuchs, 1929) Wachek, 1955.
*Stictylus pini* (Fuchs, 1929) Rühm, 1956.

S. *piceae* (Fuchs, 1929) n. comb.
Syn. *Tylenchus sulphureus piceae* Fuchs, 1929.
*Allantonema sulphureus piceae* (Fuchs, 1929) Filipjev, 1934.
*Parasitylenchus sulphureus* f. *piceae* (Fuchs, 1929) Schneider, 1939.
*Stictylus sulphureus piceae* (Fuchs, 1929) Rühm, 1956.

S. *piniphili* (Fuchs, 1929) Nickle, 1963.
Syn. *Tylenchus sulphureus piniphili* Fuchs, 1929.
*Allantonema sulphureus piniphili* (Fuchs, 1929) Filipjev, 1934.
*Parasitylenchus sulphureus* f. *piniphili* (Fuchs, 1929) Schneider, 1939.
*Stictylus piniphili* (Fuchs, 1929) Rühm, 1956.

S. *ungulacaudus* (Khan, 1957) Nickle, 1963.
Syn. *Sphaerularia ungulacauda* Khan, 1957.
*Stictylus ungulacaudus* (Khan, 1957) Khan, 1960.

This genus may eventually be placed under *Sphaerularia*, but is easily distinguished at this time by the smaller size of the prolapsed uterus, the knobbed stylet, the presence of the caudal alae. It is normally parasitic in bark beetles.

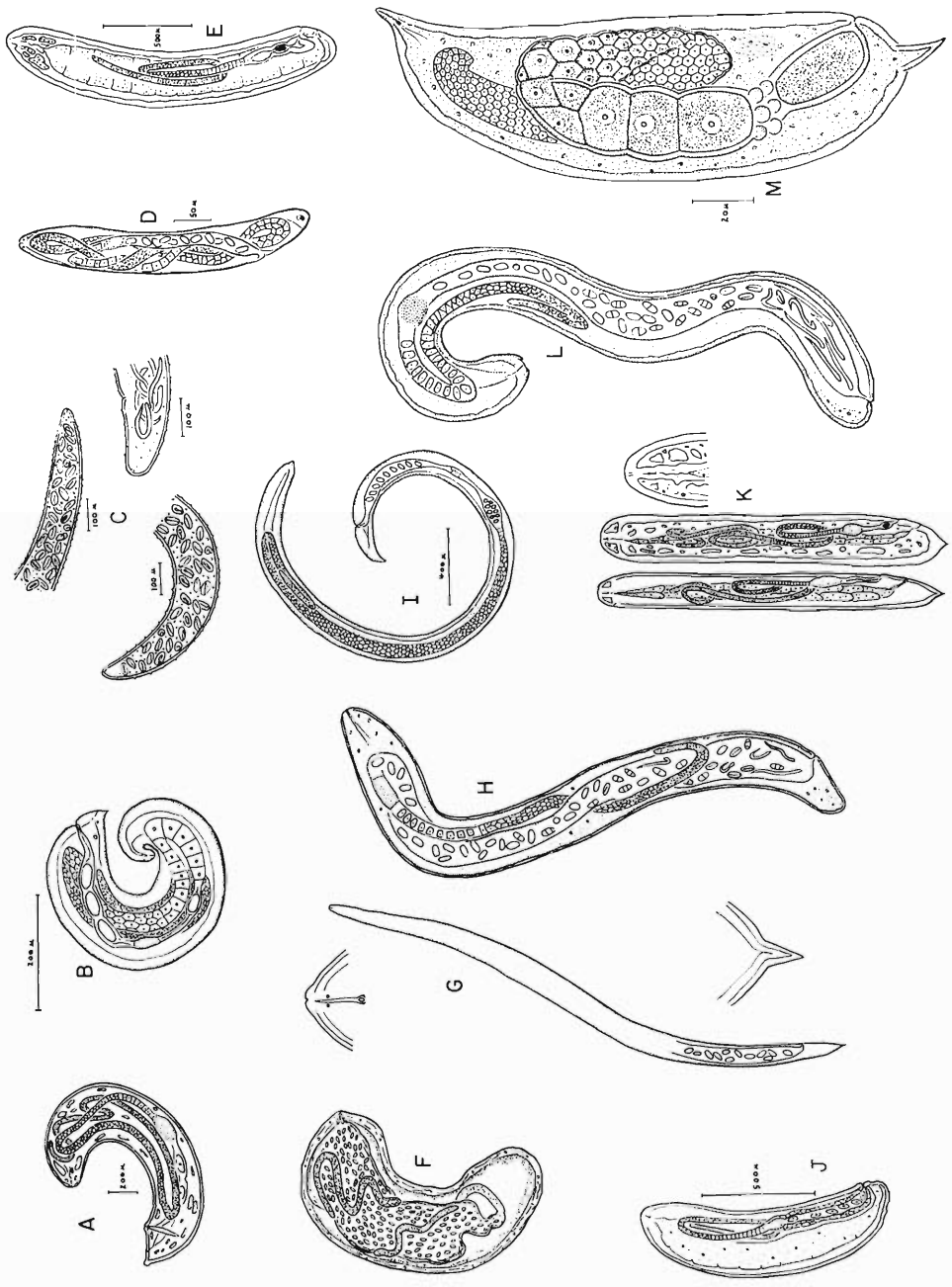



Fig. 1. Adult Parasitic Female Sphaerulariids. A. *Allantonema philonthi* (after Wachek, 1955); B. *Bovienema tomici* (after Nickle, 1963a); C. *Chondronema passali* (after Christie and Chitwood, 1931); D. *Fergusobia tumifaciens* (after Currie, 1937, in part); E. *Heterotylenchus bovieni* (after Wachek, 1955); F. *Allantonema mirabile* (after Leuckart, 1887); G. *Aphelenchulus mollis* (after Cobb, 1920, in part); H. *Bradynema rigidum*; I. *Contortylenchus elongatus* (after Nickle, 1963b); J. *Heterotylenchus stammeri* (after Wachek, 1955); K. *Protylenchus heterocerti* (after Wachek, 1955); L. *Howardula benigna*; M. *Howardula aptini* (after Nickle and Wood, 1964).

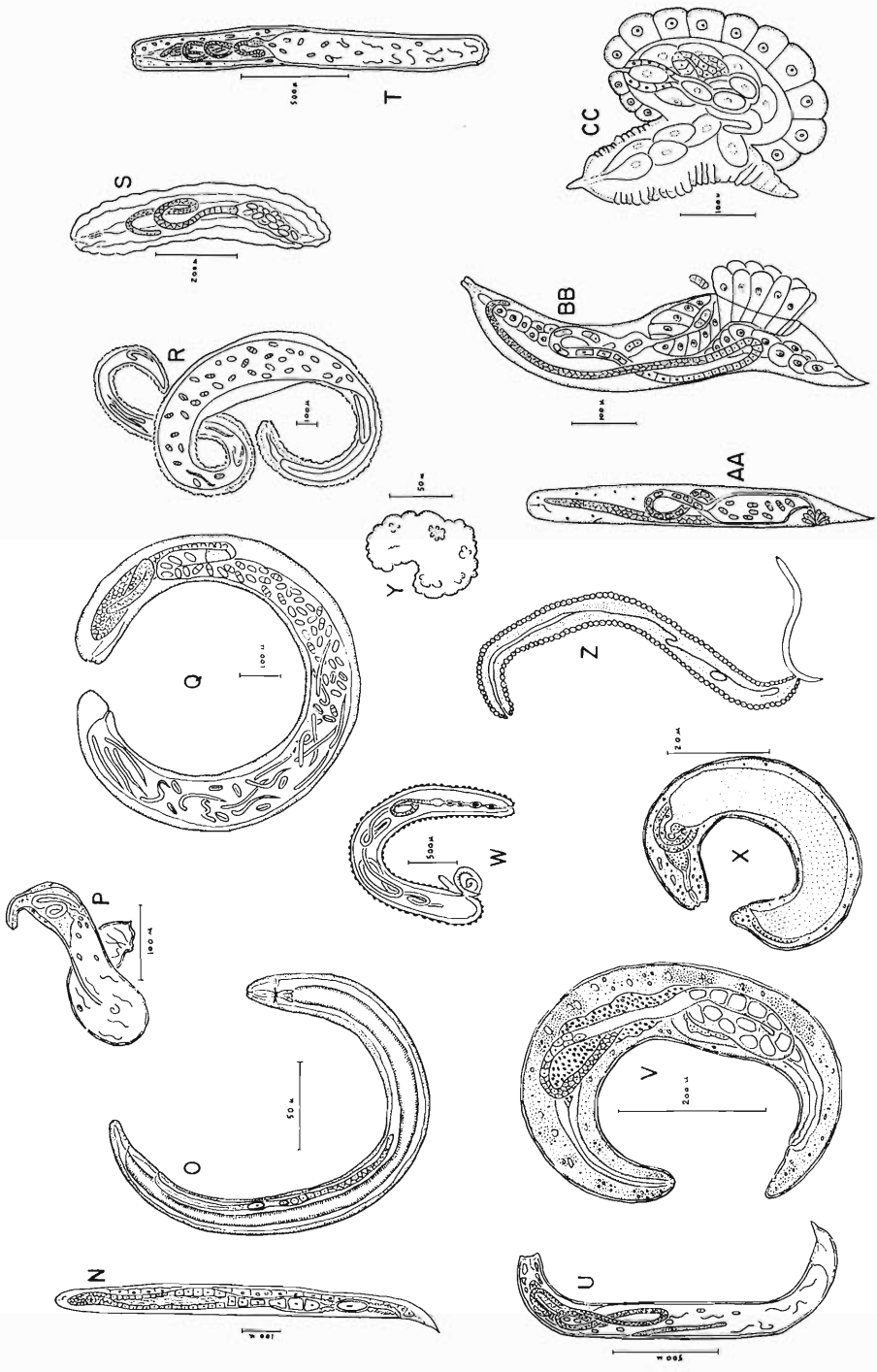



Fig. 2. Adult Parasitic Female Sphaeruiariids. N. *Heterotylenchus aberrans* (after Bovien, 1937); O. *Heterotylenchus aberrans* (after Bovien, 1937); P. *Proparasitylenchus medonis* (after Wachek, 1955); Q. *Neoparasitylenchus* sp.; R. *Sulphuretylenchus* sp.; S. *Metaparasitylenchus mycetophagi* (after Wachek, 1955); T. *Metaparasitylenchus cryptophagi* (after Wachek, 1955); U. *Parasitylenchoides paromali* (after Wachek, 1955); V. *Parasitylenchus curvidentis* (after Rühm, 1956); W. *Sphaerulariopsis stammeri* (after Wachek, 1955); X. *Parasitylenchus typographi* (after Rühm, 1956); Y. *Parasitylenchus curvidentis* (after Rühm, 1956); Z. *Sphaerularia bombi* (after Leuckart, 1887); AA. *Scatonema wilkeri* (after Bovien, 1932); BB. *Tripius sciarae* (after Bovien, 1944); CC. *Tripius gibbosus* (after Leuckart, 1887).

Genus: *Tripius* Chitwood, 1935
Syn. *Asconema* Leuckart, 1886.
*Atractonema* (Leuckart, 1886) Leuckart, 1887.
*Proatractonema* Bovien, 1944.
(Figs. 2BB, 2CC, 3Q, 3Y, 5A)

FL♀: Stylet well-developed, with knobs. Two long esophageal glands. Ovary few-celled; walls of uterus with large cells; vulva posterior. FL♂: Stylet faintly developed, without knobs. Caudal alae absent. Spicules and gubernaculum small, tylenchoid. AP♀: Fusiform. Length up to 0.9 mm. Uterus partially prolapsed. Mouth cone and tail papilla present. Stylet present, not retracted. Oviparous.

HOST INSECTS:
Diptera; *Cecidomyia* or *Dasineura*, *Bradysia*, *Sciara*.

TYPE SPECIES:
*Tripius gibbosus* (Leuckart, 1886) Chitwood, 1935.
Syn. *Asconema gibbosum* Leuckart, 1886.
*Atractonema gibbosum* (Leuckart, 1886) Leuckart, 1887.

OTHER SPECIES:
*Tripius sciara* (Bovien, 1944) Wachek, 1955.
Syn. *Proatractonema sciara* Bovien, 1944.

Poinar (1965) has shown that the morphological basis for the genus *Proatractonema* may be just a stage in the development of a *Tripius* and he considers *Proatractonema* as a synonym of *Tripius*.

SUBFAMILY:
Allantonematinae Pereira, 1931. (Emended Chitwood, 1935).
Syn. Allantoneminae Pereira, 1931.
Allantonematidae (Pereira, 1931) Chitwood and Chitwood, 1937.
Contortylenchidae Rühm, 1956.

DIAGNOSIS (Emended): Sphaerulariidae. FL♀: With or without stylet. Without eggs in uterus. FL♂: With tylenchoid anterior end. Stylet usually present, with or without knobs. Caudal alae usually peloderan when present, not voluminous. Spicules and gubernaculum tylenchoid, when present. AP♀: Swollen, uterus not everted.

Genus: *Allantonema* Leuckart, 1884
Syn. *Tylenchomorphus* Fuchs, 1915.
(Figs. 1A, 1F, 3A, 3B, 4A)

FL♀: Lips set off. Stylet well-developed. Ovary with few cells. Excretory pore posterior to nerve ring. FL♂: Lips weakly set off. Stylet present. Caudal alae narrow, peloderan. Gubernaculum small. AP♀: Bean-shaped, only 2–3 times as long as wide. Stylet retracted into body. Oviparous or ovoviviparous. Vulva terminal.

HOST INSECTS:
Coleoptera; *Hylobius*, *Hylastes*, *Philonthus*, *Ochthebius*, *Geotrupes*. Diptera; *Musca*.

TYPE SPECIES:
*Allantonema mirabile* Leuckart, 1884.
Syn. *Tylenchomorphus mirabilis* (Leuckart, 1884) Fuchs, 1915.

OTHER SPECIES:
*A. matthesi* Wachek, 1955.
*A. morosa* (Fuchs, 1929) Filipjev, 1934.
Syn. *Tylenchus morosus* Fuchs, 1929.
*Parasitylenchus morosus* (Fuchs, 1929) Filipjev and Sch. Stek., 1941.
*A. muscae* Roy and Mukherjee, 1937.
*A. philonthi* Wachek, 1955.
*A. silvaticum* von Linstow, 1892.
*A. stricklandi* Roy and Mukherjee, 1937.

It is possible that *A. muscae* and *A. stricklandi* are actually the same species, i.e., two female stages of a *Heterotylenchus*.

Genus: *Aphelenchulus* Cobb, 1920
(Figs. 1G, 3C, 4A)

FL♀: Not found. FL♂: Stylet present. Length 1 mm. Caudal alae peloderan. Gubernaculum small, trough-like. AP♀: Length 2.6 mm. Swollen, worm-shaped; tail not coiled when relaxed by heat, ventral side not turned outward. Tail with diagnostic spike; not dorsally bent, papilliform. Stylet present. Uterus containing several eggs. Oviparous.

HOST INSECT:
Coleoptera; *Cyllene*.

TYPE SPECIES:
*Aphelenchulus mollis* Cobb, 1920.

This nematode needs to be recollected and restudied to obtain additional information on its morphological relationships with various

groups in the family. The shape of the AP♀, which aids in separating this genus from *Contortylenchus*, is shown in Fig. 1G.

Genus: *Bovienema* Nickle, 1963
(Figs. 1B, 3D, 4A)

FL♀: Small. Stylet well-developed. FL♂: Stylet present. With peloderan caudal alae. Gubernaculum present. AP♀: Length 0.5–0.75 mm. Body swollen, habit in the form of a tight circle, with externally turned ventral side, see *Contortylenchus*. Tail tip characteristically peg-like, directed dorsally. Stylet always present, well-developed, not retracted. Intestine not differentiated. Oviparous. Gonad with 2 or 3 flexures, one anterior in neck region, one posterior in vicinity of vulva; one to three eggs in uterus at one time.

HOST INSECT:
Coleoptera; *Pityogenes*.

TYPE SPECIES:
*Bovienema tomici* (Bovien, 1937) Nickle, 1963.
Syn. *Aphelenchulus tomici* Bovien, 1937.
*Contortylenchus tomici* (Bovien, 1937) Rühm, 1956.

OTHER SPECIES:
*Bovienema chalcographi* (Fuchs, 1938) n. comb.
Syn. *Parasitylenchus contortus chalcographi* Fuchs, 1938.
*Parasitylenchus contortus* f. *chalcographi* (Fuchs, 1938) W. Schneider, 1939.
*Contortylenchus chalcographi* (Fuchs, 1938) Rühm, 1956.

Genus: *Bradynema* zur Strassen, 1892
(Figs. 1H, 3E, 4A)

FL♀: With smooth cuticle. Lips faintly set off. Stylet absent. Stomal region faintly cuticularized. Esophageal glands well-developed, extending almost to the genital area. Intestine narrow-celled, used for storage. FL♂: With smooth cuticle. Lips not distinctly set off. Stylet absent. Caudal alae absent or faintly developed in *B. trixagi*. Spicules tylenchoid. Gubernaculum always present. AP♀: Cuticle and hypodermis traversed by small narrow canals. Metabolic products white. Head and tail drawn in by expansion. Stylet absent. Vulva with distinct lips; ovary and oviduct short; uterus occupies up to 85% of body. Ovoviviparous.

HOST INSECTS:
Coleoptera; *Aphodius, Spondylis, Throscus*. Hemiptera; *Gerris, Velia, Nepa*. Diptera; *Bibio*.

TYPE SPECIES:
*Bradynema rigidum* (von Siebold, 1836) zur Strassen, 1892.
Syn. *Filaria rigida* von Siebold, 1836.
*Allantonema rigida* (von Siebold, 1836) Moniez, 1891.

OTHER SPECIES:
*B. bibionis* Wachek, 1955.
*B. gerridis* Poisson, 1933.
*B. nepal* Poisson, 1933.
*B. strasseni* Wülker, 1923.
*B. trixagi* Wachek, 1955.
*B. veliae* Poisson, 1933.

Genus: *Chondronema* Christie and Chitwood, 1931
(Figs. 1C, 4D)

Head with 4 distinct papillae. Stylet small, tylenchoid. Dorsal esophageal gland present. Amphidial openings lateral, slightly nearer mouth than papillae; amphidial glands large. Esophagus without bulb-like swelling. A pair of large lateral pores on the tail. Male with small caudal alae; without gubernaculum. Testis reflexed. Body of female degenerating into nearly structureless sac filled with developing embryos. Body-cavity parasites throughout larval development, but free-living throughout adult stage.

HOST INSECT:
Coleoptera; *Passalus*.

TYPE SPECIES:
*Chondronema passali* (Leidy, 1852) Christie and Chitwood, 1931.
Syn. *Nematoideum passali* Leidy, 1852.

The life history of this nematode differs from the rest of the genera in the the family because only the larval stages are parasitic in the haemocoel of the insect. No AP♀'s could be found in the body cavity of the insect. Christie and Chitwood described the adult

forms as free-living, and found them in the frass of the beetles. Larval nematodes probably leave the host during oviposition. There is no reference to the type of gonad in the female, but a small tylenchoid stylet is present. The morphology and life history of the adult forms need more study.

Genus: *Contortylenchus* Rühm, 1956
(Figs. 1I, 3F, 4A)

FL♀: Stylet usually well-developed, tip symmetrical, lumen narrow, basal knobs variable, usually prominent. Small postuterine sac. Ovary with fewer cells than in neoparasitylenchs; vulva posteriorly located, with small opening, without projecting lips. FL♂: Stylet present, dimorphic. Testis with flexure. Tail bulkier, shorter than in neoparasitylenchs; rounded, always with peloderan caudal alae, usually narrow. Spicules small, tylenchoid. Gubernaculum small. AP♀: Sulphur to brown-yellow. Always sausage-shaped or worm-shaped; often coiled when relaxed by heat, with ventral side always turned outward; the "contortus" group of Fuchs. Body surface smooth, no body swellings; anterior end often wrinkled, narrower than rest of body in the form of a movable mouth cone with a stylet. Intestine retained, well defined from rest of tissue. Gonad may have 1 or 2 flexures; oviduct longer than in neoparasitylenchs; uterus narrow, not expanded to hypodermis; vulva subterminal, deeply recessed; vagina heavily cuticularized. Anus functionless. Tail rounded, or tapering to nipple-like papilla. Oviparous.

HOST INSECTS:

Coleoptera; *Ips*, *Dendroctonus*, *Cryphalus*, *Hylastes*, *Orthotomicus*.

TYPE SPECIES:

*Contortylenchus diplogaster* (von Linstow, 1890) Rühm, 1956.

Syn. *Allantonema diplogaster* von Linstow, 1890.
*Tylenchus diplogaster* (von Linstow, 1890) Fuchs, 1915.
*Tylenchus contortus typographi* Fuchs, 1914.
*Parasitylenchus contortus typographi* (Fuchs, 1915) Micoletzky, 1922.
*Aphelenchulus contortus typographi* (Fuchs, 1915) Micoletzky, 1925.
*Anguillulina contortus typographi* (Fuchs, 1915) Baylis and Daubney, 1926.
*Aphelenchulus diplogaster* (Fuchs, 1915) Filipjev, 1934.
*Tylenchus contortus cembrae* Fuchs, 1915.
*Parasitylenchus contortus cembrae* (Fuchs, 1915) Fuchs, 1929.

OTHER SPECIES:

*C. acuminati* Rühm, 1956.

*C. amitini* Rühm, 1956.

*C. barberus* (Massey, 1957) Rühm, 1960.
Syn. *Aphelenchulus barberus* Massey, 1957.

*C. brevicomi* (Massey, 1957) Rühm, 1960.
Syn. *Aphelenchulus brevicomi* Massey, 1957.

*C. cryphali* Rühm, 1956.
Syn. *Contortylenchus cryphali* Rühm, 1954. nomen nudum.

*C. cunicularii* (Fuchs, 1929) Rühm, 1956.
Syn. *Tylenchus contortus cunicularii* Fuchs, 1929.
*Aphelenchulus cunicularii* (Fuchs, 1929) Filipjev, 1934.
*Parasitylenchus contortus* f. *cunicularii* (Fuchs, 1929) W. Schneider, 1939.

*C. elongatus* (Massey, 1960) Nickle, 1963.
Syn. *Aphelenchulus elongatus* Massey, 1960.

*C. grandicolli* (Massey, 1957) Rühm, 1960.
Syn. *Aphelenchulus grandicolli* Massey, 1957.

*C. laricis* (Fuchs, 1929) Rühm, 1956.
Syn. *Tylenchus contortus laricis* Fuchs, 1929.
*Aphelenchulus laricis* (Fuchs, 1929) Filipjev, 1934.
*Parasitylenchus contortus* f. *laricis* (Fuchs, 1929) W. Schneider, 1939.

*C. reversus* (Thorne, 1935) Rühm, 1956.
Syn. *Aphelenchulus reversus* Thorne, 1935.

*C. spirus* (Massey, 1957) Rühm, 1960.
Syn. *Aphelenchulus spirus* Massey, 1957.

The contortylenchs have the type of ovary that is typical of the family and do not differ markedly from the rest of the genera. Therefore, Contortylenchidae Rühm, 1956, is considered a synonym of Allantonematinae.

Genus: *Heterotylenchus* Bovien, 1937
(Figs. 1E, 1J, 2N, 2O, 3I, 5B)

Four distinct forms. With alternation of gamogenetic and parthenogenetic generations. FL♀: Small, slender, about 0.5 mm long. Lips faintly set off. Stylet present, with knobs. Esophagus with overlapping glands and indistinct orifices. Anus vestigial. Vulva posterior, close to anus. FL♂: Slender, about 0.5 mm long. Stylet present, small, knobbed. Tail conical. Caudal alae absent. Spicules usually small, tylenchoid. Gubernaculum lacking or very small. *Gamogenetic female* (AP♀): Yellow-brown. Body swollen, curved, inert, much larger than the free-living stage (up to 3.00 mm); both ends rounded; or, tail with spike-like papilla. Stylet present, may be retracted into body. Intestine a syncytium. Anus vestigial. Ovary, oviduct and uterus of only moderate dimensions, outstretched; or, ovary and oviduct may lie in many convolutions. Oviparous. Eggs larger than those of parthenogenetic female. *Parthenogenetic female*: More slender (up to 1.00 mm long). Anterior end drawn in by expansion. Tail conical or dome-shaped. Stylet weakly developed. Esophagus degenerate. Ovary greatly developed, reaching into head region. Oviparous or ovoviviparous; when ovoviviparous, ovary and oviduct displaced anteriorly by uterus.

HOST INSECTS:
Diptera; *Hylemya, Musca.* Coleoptera; *Bembidion, Clivina.* Siphonaptera; *Coptopsylla, Ceratophyllus.*

TYPE SPECIES:
*Heterotylenchus aberrans* Bovien, 1937.

OTHER SPECIES:
*H. bovieni* Wachek, 1955.
*H. stammeri* Wachek, 1955.
*H. wülkeri* Wachek, 1955.
*H. pawlowskyi* Kurochkin, 1960.

Genus: *Howardula* Cobb, 1921
Syn. *Tylenchinema* T. Goodey, 1930.
*Prothallonema* Christie, 1938.
*Acarinocola* Warren, 1941.
(Figs. 1L, 1M, 3J, 3K, 3L, 4A)

FL♀: Stylet present. Lips faintly set off. FL♂: Stylet absent. Caudal alae narrow, peloderan. Gubernaculum present. AP♀: Length 1.2–7.0 mm. Stylet present. White, even in old age. Head and tail retracted. Oviparous or ovoviviparous. Ovary and oviduct in oviparous species coiled within body; in ovoviviparous species, crowded together in anterior section of body. Vulva posterior, almost terminal, lips protrude slightly.

HOST INSECTS AND MITES:
Coleoptera; *Diabrotica, Phyllotreta.* Diptera; *Oscinella.* Thysanoptera; *Aptinothrips, Taeniothrips, Frankliniella.* Acarina; *Parasitus, Poecilochirus, Haemogamasus, Euryparasitus, Cosmolaelaps, Acarinocola.*

TYPE SPECIES:
*Howardula benigna* Cobb, 1921.

OTHER SPECIES:
*H. acarinorum* Wachek, 1955.
*H. aoronymphium* Welch, 1959.
*H. aptini* (Sharga, 1932) Wachek, 1955.
Syn. *Tylenchus aptini* Sharga, 1932.
*Anguillulina aptini* (Sharga, 1932) Lysaght, 1936.
*H. claviger* (Warren, 1941) Wachek, 1955.
Syn. *Acarinocola claviger* Warren, 1941.
*H. cuneifer* (Warren, 1941) Wachek, 1955.
Syn. *Acarinocola cuneifer* Warren, 1941.
*H. dubium* (Christie, 1938) Nickle, 1965.
Syn. *Prothallonema dubium* Christie, 1938.
*H. hirsuta* (Warren, 1941) Wachek, 1955.
Syn. *Acarinocola hirsutus* Warren, 1941.
*H. oscinellae* (Goodey, 1930) Wachek, 1955.
Syn. *Tylenchinema oscinellae* Goodey, 1930.
*H. phyllotretae* Oldham, 1933.
*H. terribilis* (Warren, 1941) Wachek, 1955.
Syn. *Acarinocola terribilis* Warren, 1941.

This genus contains several diverse forms, and after more study it may be separated into

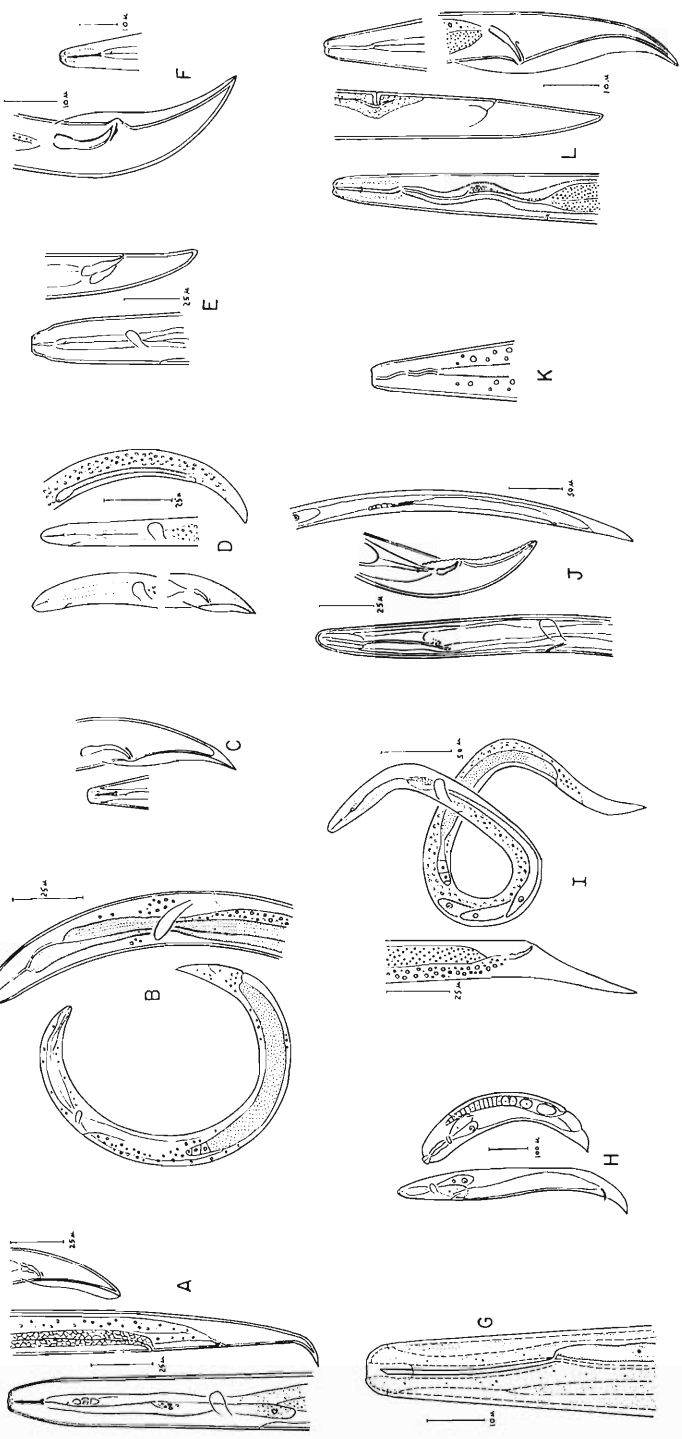
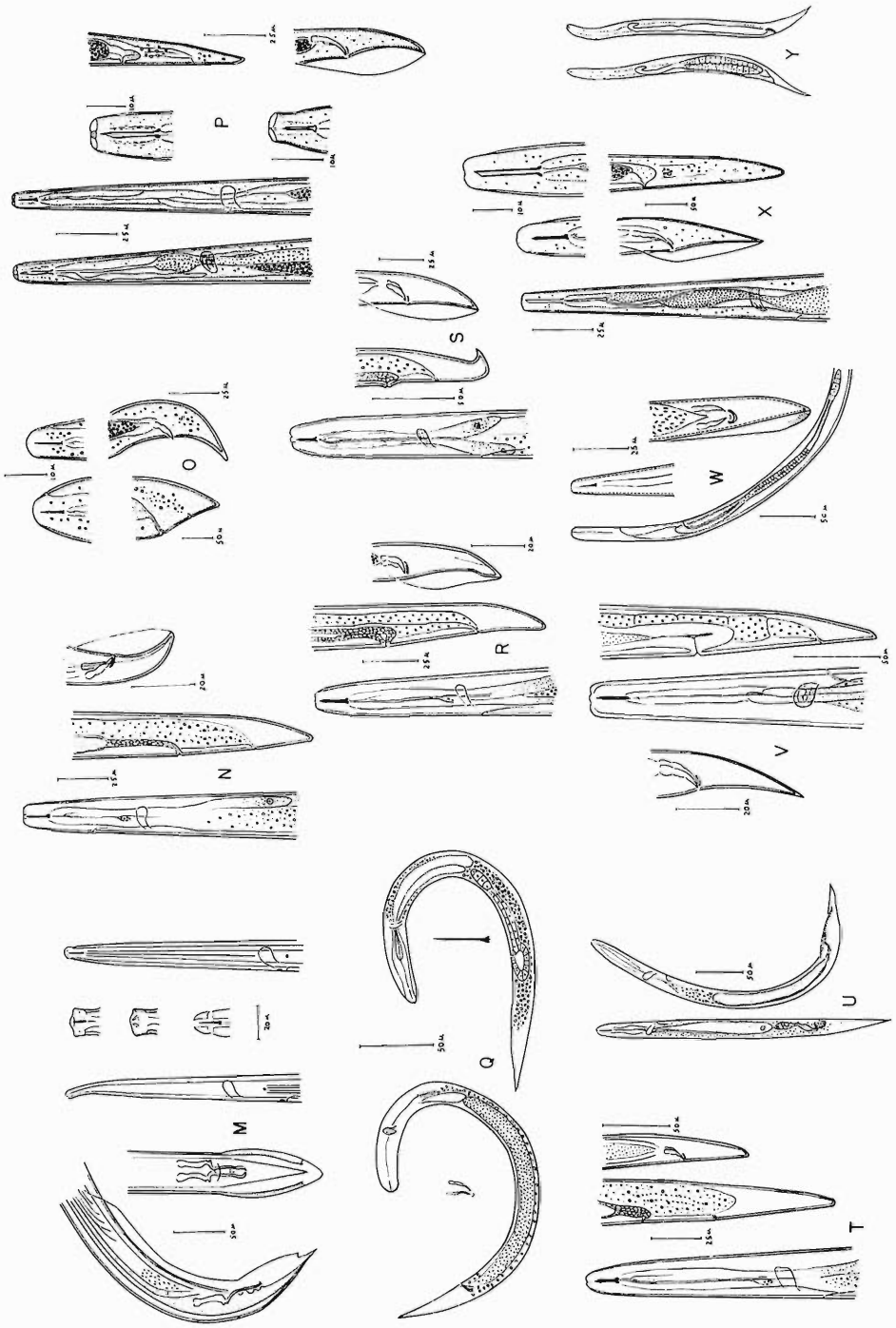


Fig. 3. Free-living Male and Female Sphaerulariids. A. *Allantonema philonthi* (after Wachek, 1955); B. *Allantonema mirabile* (after Wülker, 1923 and Bovien, 1937); C. *Aphelenchulus mollis* (after Cobb, 1920); D. *Bovienema tomici* (after Bovien, 1937); E. *Bradynema bibionis* (after Wachek, 1955); F. *Contortylenchus elongatus* (after Nickle, 1963a); G. *Dotylaphus ruehmi* (after Andrassy, 1958); H. *Fergusobia tumifaciens* (after Currie, 1937 in part); I. *Heterotylenchus aberrans* (after Bovien, 1937); J. *Howardula oscinellae* (after Goodey, 1930); K. *Howardula benigna* (Cobb's notes); L. *Howardula aptini* (after Nickle and Wood, 1964); M. *Iotonchium fungorum* (after Goodey, 1953); N. *Metaparasitylenchus mycetophagi* (after Wachek, 1955); O. *Parasitylenchus typographi* (after Rühm, 1956); P. *Neoparasitylenchus cryphali* (after Rühm, 1956); Q. *Tripius sciarae* (after Bovien, 1944); R. *Proparasitylenchus medonis* (after Wachek, 1955); S. *Parasitylenchoides paromali* (after Wachek, 1955); T. *Protylenchus heteroceri* (after Wachek, 1955); U. *Scatonema wilkeri* (after Bovien, 1932); V. *Sphaerularia bombi* (after Wachek, 1955, in part); W. *Sphaerulariopsis hastatus* (after Khan, 1957a); X. *Sulphuretylenchus grossmannae* (after Rühm, 1954); Y. *Tripius gibbosus* (after Leuckart, 1887).



valid genera or subgenera. The distinguishing feature is the lack of stylet in the male.

Genus: *Metaparasitylenchus* (Wachek, 1955) n. grad.
(Figs. 2S, 2T, 3N, 4A)

Larvae leave host after second moult, third moult occurs in free-life. After copulation, impregnated young females infect host larva or pupa, moult once in new host for fourth and last time (after Wachek, 1955). FL♀: Stylet strongly knobbed. FL♂: Stylet present. Caudal alae peloderan, frequently broad. Gubernaculum present. AP♀: White, seldom with yellow-brown deposits. Oviparous or ovoviviparous.

HOST INSECTS:

Coleoptera (not bark beetles); *Cryptophagus, Mycetophagus, Telmatophilus, Cossonus, Tetropium, Strangalia, Rhizophagus, Helmis, Latelmis, Riolus.*

TYPE SPECIES:

*Metaparasitylenchus telmatophili* (Wachek, 1955) n. grad.
Syn. *Parasitylenchus* (*Metaparasitylenchus*) *telmatophili* Wachek, 1955.

OTHER SPECIES:

*M. cossoni* (Wülker, 1929) n. comb.
Syn. *P. cossoni* Wülker, 1929.
*P.* (*M.*) *cossoni* (Wülker, 1929) Wachek, 1955.
*M. cryptophagi* (Wachek, 1955) n. comb.
Syn. *P.* (*M.*) *cryptophagi* Wachek, 1955.
*M. helmidis* (Wachek, 1955) n. comb.
Syn. *P.* (*M.*) *helmidis* Wachek, 1955.
*M. mycetophagi* (Wachek, 1955) n. comb.
Syn. *P.* (*M.*) *mycetophagi* Wachek, 1955.
*M. oschei* (Rühm, 1956) n. comb.
Syn. *P.* (*M.*) *oschei* Rühm, 1956.
*M. rhizophagi* (Wachek, 1955) n. comb.
Syn. *P.* (*M.*) *rhizophagi* Wachek, 1955.
*M. strangaliae* (Wachek, 1955) n. comb.
Syn. *P.* (*M.*) *strangaliae* Wachek, 1955.
*M. tetropii* (Wachek, 1955) n. comb.
Syn. *P.* (*M.*) *tetropii* Wachek, 1955.

Genus: *Neoparasitylenchus* n. gen.
(Figs. 2Q, 3P, 4A)

With 3 distinct forms; one free-living sexual generation, one swollen female developing from the mated free-living female. FL♀: Stylet well-developed, with basal swellings. Ovary with more cells than *Contortylenchus* and *Allantonema* spp. FL♂: Stylet present, not prominent. Caudal alae peloderan or leptoderan. Spicules and gubernaculum small, tylenchoid. AP♀: Swollen, slightly mobile, usually sausage- or worm-shaped. Stylet not retracted into inner body. Tail terminal with or without papilla. Oviparous or ovoviviparous. Ovary and oviduct intertwined irregularly, crowded together anteriorly; uterus long, wide, filled with eggs or larvae.

HOST INSECTS:

Coleoptera (bark beetles); *Hylurgus, Hylastes, Dendroctonus, Scolytus, Cryphalus, Crypturgus, Pityophthorus, Pityogenes, Ips.*

TYPE SPECIES:

*Neoparasitylenchus cryphali* (Fuchs, 1914) n. comb.
Syn. *Tylenchus dispar cryphali* Fuchs, 1914.
*Parasitylenchus dispar* var. *cryphali* (Fuchs, 1914) Micoletzky, 1922.
*Aphelenchulus cryphali* (Fuchs, 1914) Filipjev, 1934.
*Parasitylenchus dispar* f. *cryphali* (Fuchs, 1914) Schneider, 1939.
*Parasitylenchus* (*Parasitylenchus*) *cryphali* (Fuchs, 1914) Rühm, 1956.

OTHER SPECIES:

*N. avulsi* (Massey, 1958) n. comb.
Syn. *Parasitylenchus avulsi* Massey, 1958.
*P.* (*P.*) *avulsi* (Massey, 1958) Rühm, 1960.
*N. betulae* (Rühm, 1956) n. comb.
Syn. *P.* (*P.*) *betulae* Rühm, 1956.
*N. chalcographi* (Fuchs, 1938) n. comb.
Syn. *P. dispar chalcographi* Fuchs, 1938.
*P. dispar* f. *chalcographi* (Fuchs, 1938) Schneider, 1939.
*P. chalcographi* (Fuchs, 1938) Filipjev & Sch. Stek., 1941.
*P.* (*P.*) *chalcographi* (Fuchs, 1938) Rühm, 1956.
*N. cinerei* (Fuchs, 1929) n. comb.
Syn. *Tylenchus dispar cinerei* Fuchs, 1929.
*Aphelenchulus cinerei* (Fuchs, 1929) Filipjev, 1934.
*P. dispar* f. *cinerei* (Fuchs, 1929) Schneider, 1939.

*P.* (*P.*) *cinerei* (Fuchs, 1929) Rühm, 1956.
*P. dispar pusilli* Fuchs, 1938.
*P. dispar* f. *pusilli* (Fuchs, 1938) Schneider, 1939.
*P. pusilli* (Fuchs, 1938) Filipjev and Stek., 1941.

*N. hylastis* (Wülker, 1923) n. comb.
Syn. *Tylenchus hylastis* Wülker, 1923.
*Tylenchus dispar ateri* Fuchs, 1929.
*Tylenchus dispar cunicularii* Fuchs, 1929.
*P. hylurgi* Filipjev, 1934.
*P. dispar* f. *cunicularii* (Fuchs, 1929) Schneider, 1939.
*P. dispar* f. *ateri* (Fuchs, 1929) Schneider, 1939.
*P. hylastis* (Wülker, 1923) Filipjev, 1934.
*P.* (*P.*) *hylastis* (Wülker, 1923) Rühm, 1956.

*N. ligniperdae* (Fuchs, 1929) n. comb.
Syn. *Tylenchus ligniperdae* Fuchs, 1929.
*P. ligniperdae* (Fuchs, 1929) Filipjev, 1934.
*P.* (*P.*) *ligniperdae* (Fuchs, 1929) Rühm, 1956.

*N. orthotomici* (Rühm, 1960) n. comb.
Syn. *P.* (*P.*) *orthotomici* Rühm, 1960.

*N. ovarius* (Massey, 1958) n. comb.
Syn. *P. ovarius* Massey, 1958.

*N. pessoni* (Rühm, 1957) n. comb.
Syn. *P.* (*P.*) *pessoni* Rühm, 1957.

*N. pityophthori* (Rühm, 1956) n. comb.
Syn. *P.* (*P.*) *pityophthori* Rühm, 1956.

*N. poligraphi* (Fuchs, 1938) n. comb.
Syn. *P. dispar poligraphi* Fuchs, 1938.
*P. dispar* f. *poligraphi* (Fuchs, 1938) Schneider, 1939.
*P. poligraphi* (Fuchs, 1938) Filipjev and Sch. Stek., 1941.
*P.* (*P.*) *poligraphi* (Fuchs, 1938) Rühm, 1956.

*N. rugulosi* (Schvester, 1957) n. comb.
Syn. *P. dispar rugulosi* Schvester, 1957.
*P.* (*P.*) *rugulosi* (Schvester, 1957) Rühm, 1960.

*N. scolyti* (Oldham, 1930) n. comb.
Syn. *P. scolyti* Oldham, 1930.
*P. secundus* Fuchs, 1933.
*P.* (*P.*) *scolyti* (Oldham, 1930) Rühm, 1956.

*N. wuelkeri* (Rühm, 1956) n. comb.
Syn. *P.* (*P.*) *wuelkeri* Rühm, 1956.

*N. xylebori* (Schvester, 1950) n. comb.
Syn. *P. dispar xylebori* Schvester, 1950.
*P.* (*P.*) *xylebori* (Schvester, 1950) Rühm, 1956.

Genus: *Parasitylenchoides* Wachek, 1955
(Figs. 2U, 3S, 4A)

FL♀: Excretory pore 0.105–0.125 mm from anterior end, against 0.038–0.072 mm in *Neoparasitylenchus* (Diagnostic). Stylet present, with knobs. FL♂: Stylet present. Caudal alae peloderan or absent. Spicules tylenchoid. Gubernaculum small. AP♀: Swollen, worm-shaped; head and tail not drawn in by expansion; with mouth-cone and tail papilla. Stylet in normal position or retracted. Oviparous or ovoviviparous. With oviparity, ovary and oviduct show few windings. With ovoviviparity, ovary displaced anteriorly or to the side as uterus fills 80% of body. Metabolic products yellow-brown.

HOST INSECTS:
Coleoptera; *Anobium, Plegaderus, Micromalthus, Ditoma, Paederus, Sciodrepa, Aleochara, Oxytelus, Stenus, Paromalus.*

TYPE SPECIES:
*P. steni* Wachek, 1955.

OTHER SPECIES:
*P. anobii* Wachek, 1955.
*P. diatomae* Wachek, 1955.
*P. körneri* Wachek, 1955.
*P. paederi* Wachek, 1955.
*P. paromali* Wachek, 1955.
*P. rheocharae* Wachek, 1955.
*P. sciodrepae* Wachek, 1955.
*P. wichmanni* Wachek, 1955.

Genus: *Parasitylenchus* Micoletzky, 1922
Syn. *Polymorphotylenchus* Rühm, 1956.
(Figs. 2V, 2X, 2Y, 3O, 4C)

With 5 distinct forms, 2 sexual generations, one swollen female developing from mated free-living female. *Small parasitic female:* Active, narrow, sausage-shaped, present in large numbers; or motionless, yellow-brown, raisin-shaped. Stylet present, may be retracted, smaller than in large female; basal knobs small, flat. Gonad with a short flexure, uterus with few eggs; or greatly developed with nu-

merous flexures of ovary. *Small parasitic male:* Active, narrow. Lips not set off. Excretory pore opens near stylet. Stylet present, weakly developed. Esophagus and intestine weak. Gonad outstretched, extends almost to stylet. Tail plump, rounded. Caudal alae present or absent. Spicules and gubernaculum small. *Free-living stages not described.* AP♀: Swollen, sausage-shaped, inwardly curved, milky white with red-brown spots; may be transparent or partly dark brown. Stylet present, knobs very small, flat. Gonad well-developed; ovary and oviduct with numerous crowded loops in anterior end; uterus large; or gonad weakly developed with more or less long flexure, not pushed anteriorly, uterus narrow with few eggs.

HOST INSECTS:

Coleoptera; *Ips, Pityokteines.* Diptera; *Drosophila.*

TYPE SPECIES:

*Parasitylenchus dispar* (Fuchs, 1915) Filipjev, 1934.

Syn. *Tylenchus dispar typographi* Fuchs, 1914. nomen nudum.
*Tylenchus dispar typographi* Fuchs, 1915.
*Parasitylenchus dispar* var. *typographi* (Fuchs, 1915) Micoletzky, 1922.
*Aphelenchulus dispar* var. *typographi* (Fuchs, 1915) Micoletzky, 1925.
*Anguillulina dispar* var. *typographi* (Fuchs, 1915) Baylis and Daubney, 1926.
*Parasitylenchus dispar* f. *typographi* (Fuchs, 1915) Schneider, 1939.
*Polymorphotylenchus* (*Polymorphotylenchus*) *typographi* (Fuchs, 1915) Rühm, 1956.

OTHER SPECIES:

*Parasitylenchus curvidentis* (Fuchs, 1914) Micoletzky, 1922.

Syn. *Tylenchus dispar curvidentis* Fuchs, 1914.
*Parasitylenchus dispar* var. *curvidentis* (Fuchs, 1914) Micoletzky, 1922.
*Aphelenchulus dispar* var. *curvidentis* (Fuchs, 1914) Micoletzky, 1925.
*Anguillulina dispar* var. *curvidentis* (Fuchs, 1914) Baylis and Daubney, 1926.
*Aphelenchulus curvidentis* (Fuchs, 1914) Filipjev, 1934.
*Parasitylenchus dispar* f. *curvidentis* (Fuchs, 1914) Schneider, 1939.
*Polymorphotylenchus* (*Thylakolenchus*) *curvidentis* (Fuchs, 1915) Rühm, 1956.

*Parasitylenchus diplogenus* Welch, 1959.

Syn. *Polymorphotylenchus diplogenus* (Welch, 1959) Baker, 1962.

Welch (1959) pointed out that Rühm improperly used the type species of the genus *Parasitylenchus* Micoletzky, 1922, as the type species of his new genus *Polymorphotylenchus.* Filipjev's 1934 designation of *Tylenchus dispar typographi* Fuchs, 1915 as the only synonym of his type species, *Parasitylenchus dispar* (Fuchs, 1915) may not have been the best choice; however, it is valid according to the International Rules of Zoological Nomenclature. Therefore, most of the other species have been removed from *Parasitylenchus* and placed in a new genus *Neoparasitylenchus.* The subgenera *Sulphuretylenchus, Metaparasitylenchus,* and *Proparasitylenchus* have been raised to generic level. *Polymorphotylenchus* has been synonymized with *Parasitylenchus.*

## Genus: *Proparasitylenchus* Wachek, 1955 n. grad.
(Figs. 2P, 3R, 4A)

FL♀: Copulated females penetrate into host pupa. Stylet present, usually with strong knobs, never bare or cleft. FL♂: Stylet present. Caudal alae peloderan or leptoderan, always present. Gubernaculum present. AP♀: White, or faintly citron-yellow, never brown. Ovoviviparous. Larvae leave host after second moult, two moults in free-life.

HOST INSECT:

Coleoptera (staphylinids); *Platystethus, Medon, Trogophloeus, Zyras, Atheta, Oxytelus.*

TYPE SPECIES:

*Proparasitylenchus platystethi* (Wachek, 1955) n. grad.

Syn. *Parasitylenchus* (*Proparasitylenchus*) *platystethi* Wachek, 1955.

OTHER SPECIES:

*P. athetae* (Wachek, 1955) n. grad.
Syn. *P.* (*P.*) *athetae* Wachek, 1955.

*P. boopini* (Wachek, 1955) n. grad.
Syn. *P.* (*P.*) *boopini* Wachek, 1955.

*P. medonis* (Wachek, 1955) n. grad.
Syn. *P.* (*P.*) *medonis* Wachek, 1955.

*P. myrmedoniae* (Wachek, 1955) n. grad.
Syn. *P.* (*P.*) *myrmedoniae* Wachek, 1955.

*P. oxyteli* (Wachek, 1955) n. grad.
Syn. *P.* (*P.*) *oxyteli* Wachek, 1955.

*P. trogophloei* (Wachek, 1955) n. grad.
Syn. *P.* (*P.*) *trogophloei* Wachek, 1955.

Genus: *Protylenchus* Wachek, 1955
(Figs. 1K, 3T, 4A)

FL♀: Lips not set off. Stylet present. FL♂: Lips not set off. Stylet well-developed with three cleft knobs. Caudal alae absent. Spicules tylenchoid. Gubernaculum proximally thickened. AP♀: Cuticle bare, hypodermis thick. Stylet present, retracted within body. Tail papilla present. Ovary and oviduct long, coiled profusely throughout body; vulval lips slightly arched forward; uterus short. Oviparous. Metabolic products white. No alternation of generations.

HOST INSECT:

Coleoptera; *Heterocerus*.

TYPE SPECIES:

*P. heteroceri* Wachek, 1955.

Genus: *Sulphuretylenchus* Rühm, 1956 n. grad.
(Figs. 2R, 3X, 4A)

FL♀: Longer than most sphaerulariids. V–A distance longer than neoparasitylenchs. Excretory pore opens below nerve ring. Stylet large; lumen wide. FL♂: Stylet present. Tail longer than typical neoparasitylench. Caudal alae peloderan. Spicules and gubernaculum small. AP♀: Shape variable, usually long, with swollen spot on tube-shaped body, swelling caused by congestion of larvae and eggs (Endotokie matricide). Body surface characteristically wavy, with constrictions. Sulphur to yellow-brown or dark brown. Stylet retracted into inner body, spear tip asymmetrical, basal swellings flat, not knobbed. Esophagus and intestine degenerate. Ovoviviparous. Vulva and anus difficult to see in mature parasites. Gonad almost fills body, flexed about 3 times. Papillate tail tip absent.

HOST INSECTS:

Coleoptera (bark beetles); *Pityogenes, Ips, Polygraphus, Dryocoetes, Hylastes, Scolytus.*

TYPE SPECIES:

*Sulphuretylenchus sulphureus* (Fuchs, 1938) n. grad.
Syn. *Parasitylenchus sulphureus chalcographi* Fuchs, 1938.
*Parasitylenchus* (*Sulphuretylenchus*) *sulphureus* (Fuchs, 1938) Rühm, 1956.

OTHER SPECIES:

*S. elongatus* (Massey, 1958) n. comb.
Syn. *P. elongatus* Massey, 1958.

*S. escherichi* (Rühm, 1956) n. grad.
Syn. *P.* (*S.*) *escherichi* Rühm, 1956.

*S. fuchsi* (Fuchs, 1938) n. grad.
Syn. *P. sulphureus poligraphi* Fuchs, 1938.
*P.* (*S.*) *fuchsi* (Fuchs, 1938) Rühm, 1956.

*S. grossmannae* (Rühm, 1954) n. grad.
Syn. *Paratylenchus* (*Sulphuretylenchus*) *grossmannae* Rühm, 1954. Lapsus calami.

*S. kleinei* (Rühm, 1956) n. grad.
Syn. *Parasitylenchus* (*Sulphuretylenchus*) *kleinei* Rühm, 1956.

*S. pilifronis* (Massey, 1958) n. comb.
Syn. *P. pilifronis* Massey, 1958.

The significance of a group of genera with a long stylet, without knobs, and with a dorsal gland orifice which opens over a stylet length behind the stylet base, is not known to me at this time. It includes some species of *Sulphuretylenchus, Howardula, Heterotylenchus* and others. Future taxonomic work will undoubtedly clarify this relationship.

Genus: *Scatonema* Bovien, 1932
(Figs. 2AA, 3U, 4A)

FL♀: Small (0.33 mm long), slender. Stylet well-developed, with basal knobs; tip with oblique cut. First part of esophagus with distinct cuticular lining; two large ventral esophageal glands open separately into esophagus in

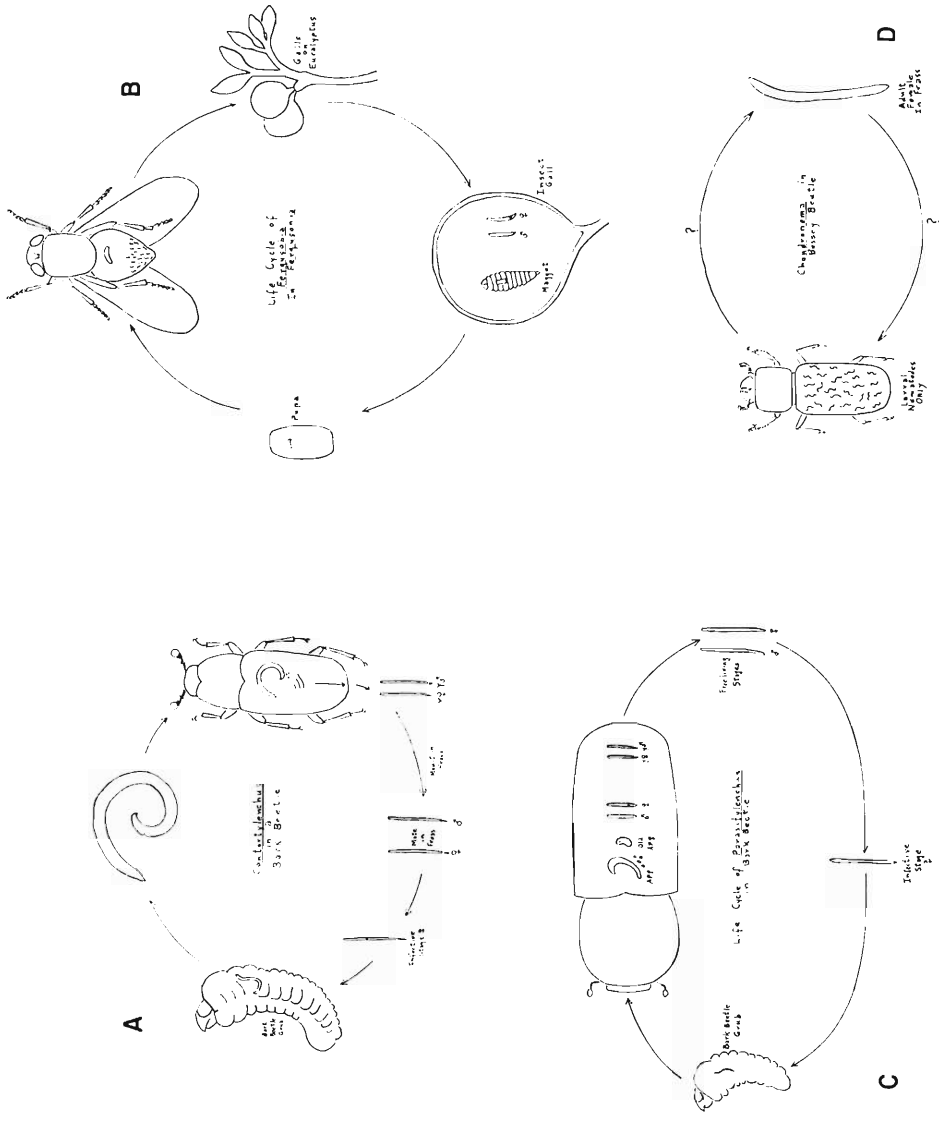



Fig. 4. Life cycle of Sphaerulariids. A. Simple Type; B. Eggs in Free-living Female; C. Two Sexual Generations; D. Only Larval Stages in Body Cavity.

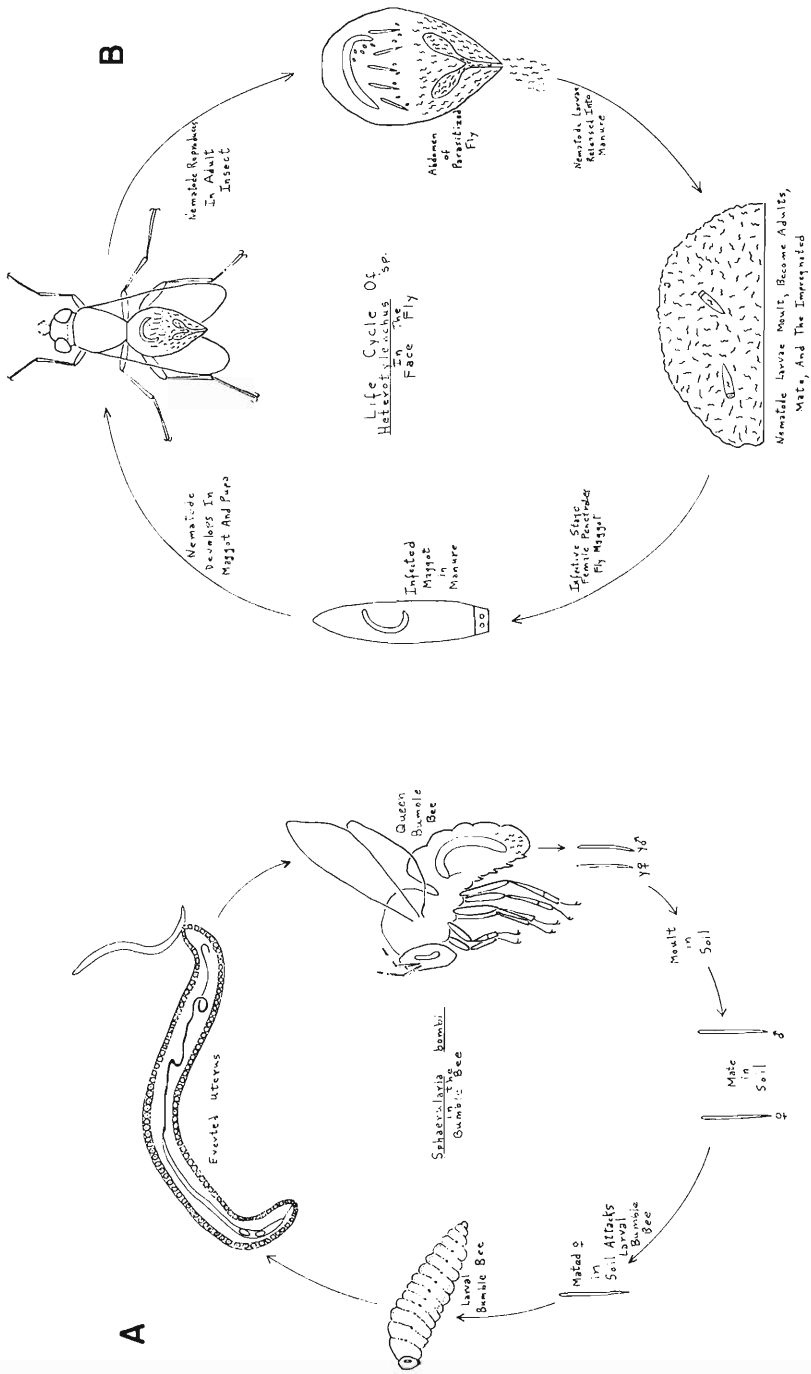



Fig. 5. Life Cycles of Sphaerulariids. A. Everted Uterus; B. Endoparasitic Parthenogenetic Generation.

front of nerve ring. No anal opening found. Oocytes few. Vagina surrounded by circle of large perivaginal cells. Tail tapering posteriorly. FL♂: Small (0.33 mm long), slender. Stylet absent. Esophageal glands not seen. Tail tapering behind anus. Spicules and gubernaculum tylenchoid. Caudal alae present, narrow. AP♀: Cigar- or sausage-shaped. Often coiled. Ovary greatly developed; swelling at end of oviduct serves as receptaculum seminis; uterus occupies bulk of body; perivaginal cells strikingly developed. Ovoviviparous.

HOST INSECT:

Diptera; *Scatopse*.

TYPE SPECIES:

*Scatonema wülkeri* Bovien, 1932.

Genus: *Dotylaphus* Andrassy, 1958
(Fig. 3G)

Based on one specimen. FL♀: Cuticle annulated. Head not set-off, with small lips and papillae. Stylet short, well-developed, similar to species of *Dorylaimus*, however, tip slanting ventrally; guiding ring asymmetrical; stylet knobs are lacking; stylet continuation sclerotized, relatively long; lumen wide (similar to *Dorylaimus*). Esophagus without muscles, without bulb, opening gradually into intestine; esophageal glands well-developed, long, lie free in body cavity, outstretched, with enlarged ampulla-like openings; subventral gland orifices in the middle of esophagus; dorsal gland orifice between anterior end and middle of esophagus, far behind stylet. Lateral organs not seen. Lateral field well-developed, with several lines. Gonad single, prodelphic; vulva is posteriorly located. Tail short, rounded. FL♂: Unknown. AP♀: Unknown.

HOST INSECT:

Unknown.

TYPE SPECIES:

*Dotylaphus rühmi* Andrassy, 1958.

This specimen belongs in this family. Unfortunately, it can fit into a few existing genera and without further information it is difficult to place at this time. Therefore, these taxa should be treated as *genus* and *species inquirendae*.

SUBFAMILY:

Fergusobiinae J. B. Goodey, 1963.

Syn. Fergusobiidae Siddiqi and J. B. Goodey, 1963.

DIAGNOSIS (Emended): Sphaerulariidae. FL♀: Found in Eucalyptus gall. Eggs in uterus. Stylet well-developed. FL♂: With tylenchoid anterior end. Stylet present, knobbed. Caudal alae peloderan, not voluminous. Spicules tylenchoid. Gubernaculum absent. AP♀: Swollen, uterus not everted.

Genus: *Fergusobia* Currie, 1937

Syn. *Anguillulina* (*Fergusobia*) Currie, 1937.

(Figs. 1D, 3H, 4B)

Three stages found: FL♀: In gall. Head offset, narrower than body, which is quite plump and faintly annulated. Spear tylenchoid with well-developed basal knobs. Anterior part of esophagus distended by ampullae filled with esophageal secretions and appearing valve-like at times; esophagus narrows considerably at isthmus surrounded by nerve ring; glands overlap the intestine; dorsal gland orifice typically tylenchoid, just behind spear base; subventral esophageal glands empty into swollen median bulb-like area. Excretory pore posterior to nerve ring. Female gonad prodelphic; no postuterine sac; vulva with distinct lips. Eggs present. Rectum and anus visible. FL♂: Spicules paired, robust, knobbed, arcuate. Gubernaculum absent. Goodey illustrates a narrow, peloderan caudal alae. Stylet present. Gonad single, outstretched. Tails of both sexes short, conoid, rounded. AP♀: Similar in shape to *Heterotylenchus* from carabid beetles. Oviparous. Ovary winds several times within body. Stylet retracted. Numerous lateral somatic setae present.

HOST INSECT:

Diptera; *Fergusonina*.

TYPE SPECIES:

*Fergusobia tumifaciens* (Currie, 1937) Chitwood and Chitwood, 1950.

Syn. *Anguillulina* (*Fergusobia*) *tumifaciens* Currie, 1937.

*Anguillulina* (*Fergusobia*) *curriei* (Currie, 1937) Johnston, 1938.

I have studied males and females from the gall and also the adult parasitic females from the body cavity of the insect. Though the subventral gland orifices are swollen in the middle of the esophagus, no criconematid valve could be found. This fact dismisses any possible association with the Criconematidae and dis-

allows the family Fergusobiidae Siddiqi and J. B. Goodey, 1963. Fisher (1965) has recently found that the female in the gall produces eggs. This separates this genus from all other members of this family.

SUBFAMILY:

Iotonchiinae T. Goodey, 1953.

Syn. Iotonchiidae (T. Goodey, 1953) Skarbilovich, 1959.

DIAGNOSIS (Emended): Sphaerulariidae. FL ♀: With post-vulval and mid-ventral supplement. Without eggs. Stylet well-developed. FL ♂: Head bilaterally symmetrical, tri-lobed, flattened dorso-ventrally. Stylet not well-developed. Spicules large, L-shaped, elaborate. Caudal alae voluminous, envelop tail tip. Post anal papillae sometimes present. AP ♀: Unknown.

## Genus: *Iotonchium* Cobb, 1920 (Fig. 3M)

Esophagus ill-defined; dorsal gland orifice joining esophageal lumen 4 head widths from anterior end of body; subventral esophageal glands opening about 4–5 head-widths further back; anterior part of esophagus cylindrical, nerve ring crossing slightly constricted isthmus; posterior part not distinguishable from intestine. Lateral fields, deirids and hemizonid present in both sexes. Female: Spear well-developed, probably changing shape at final moult. Male: Head offset by expansion, stylet not well-developed. Spicules large, markedly angular and L-shaped, sometimes with posterior extensions protruding through cloaca. Gubernaculum absent. Pre-adult male larva precocious; having fully developed spicules and gonad but no caudal alae. Head of larval male radially symmetrical, changing to adult shape at final moult. AP ♀: Unknown.

HOST INSECT:

Unknown. Found in basidiomycetous fungi *Entoloma*, *Pleurotus*, *Hygrophorus*, and *Tricholoma*.

TYPE SPECIES:

*Iotonchium imperfectum* (Bütschli, 1876) Cobb, 1920.

Syn. *Tylenchus imperfectus* Bütschli, 1876.

*Anguillulina* (*Iotonchium*) *imperfecta* (Bütschli, 1876) W. Schneider, 1939.

OTHER SPECIES:

*I. fungorum* (Bütschli, 1873) Fil. and Stek., 1941.

Syn. *Tylenchus fungorum* Bütschli, 1873.
*Hexatylus fungorum* (Bütschli, 1873) T. Goodey, 1932.
*Tylenchus* (*Anguillulina*) *fungorum* (Bütschli, 1873) Filipjev, 1934.
*Neotylenchus fungorum* (Bütschli, 1873) Filipjev, 1936.
*Anguillulina fungorum* (Bütschli, 1873) W. Schneider, 1939.

*I. bifurcatum* T. Goodey, 1953.

*I. cephalostrictum* Meyl, 1954.

*I. macrospiculatum* (Meyl, 1954) J. B. Goodey, 1956.

Syn. *Hexatylus macrospiculatus* Meyl, 1954.

*I. mycophilum* Meyl, 1954.

T. Goodey (1953) and J. B. Goodey (1956, 1963) showed the correct sphaerulariid status of *Iotonchium*. I also agree with the synonymy of Iotonchiidae (T. Goodey, 1953) Skarbilovich, 1959, with Iotonchiinae T. Goodey, 1953. In so doing we may discount Bütschli's female of *Tylenchus imperfectus* (His Fig. 7d) which showed an egg in the female, and accept the sphaerulariid ovary as described by T. Goodey. However, the female with the egg may be valid and could represent a situation similar to that of *Fergusobia*. The life cycles of these nematodes need more study, especially the identity of the host insects, if any, and the descriptions of AP ♀'s.

## SUMMARY

A taxonomic revision of the insect parasitic nematode family Sphaerulariidae Lubbock, 1861 is presented and includes 4 subfamilies, 21 genera, and 116 species. The family group diagnoses are emended, and generic diagnoses are given along with a listing of species and synonymies. The dorsal esophageal gland orifice of *Sphaerularia bombi* was found for the first time and was located in the normal tylenchoid position. This discovery required the shifting of the Sphaerulariidae from the Aphelenchoidea to the Tylenchoidea, and because the Sphaerulariidae is an older family group name than the Allantonematidae the latter drops to subfamily status. *Polymorphotylenchus* Rühm, 1956 is synonymized with

*Parasitylenchus* Micoletzky, 1922 and *P. dispar* (Fuchs, 1915) Filipjev, 1934 is considered as the type species of *Parasitylenchus*. The remaining species of the old genus *Parasitylenchus* are placed in a new genus *Neoparasitylenchus*, requiring 16 new combinations. The families Contortylenchidae and Allantonematidae are considered at this time to be synonyms of the Allantonematinae. Fergusobiidae is synonymized with Fergusobiinae which is placed as a subfamily of the Sphaerulariidae. The writer agrees with Goodey's synonymy of Iotonchiidae with Iotonchiinae and its placement with these insect parasites. *Dotylaphus rühmi* Andrassy, 1958 is treated as *genus* and *species inquirendae*. The subgenera *Sulphuretylenchus* Rühm, 1956, *Metaparasitylenchus* Wachek, 1955, and *Proparasitylenchus* Wachek, 1955 are raised to generic rank. Host insects are given whenever possible because they are useful in identification. Typical life cycles are illustrated along with plates of representative adult parasitic females, and free-living male and female stages.

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## Encholaimoidea (Nematoda: Dorylaimida), A New Superfamily Representing Dorylaimid Specimens with Cephalic Setae

A. MORGAN GOLDEN[1] AND D. G. MURPHY[1]

In the fall of 1963 nematodes obtained from a soil sample around the roots of coconut (*Cocos nucifera* L.) in Trinidad were submitted for identification by Dr. Karl Maramorosch, Boyce Thompson Institute for Plant Research, Yonkers, New York. Among the nematodes found in this and other samples collected shortly thereafter from the same area were a total of about 30 specimens of a very remarkable nematode. The amphids, spear, esophagus, and certain other structures were clearly dorylaimid. Furthermore, the development of the spear as seen in immature specimens, is the same as in other species of spear-bearing dorylaims. Yet, these specimens possess four small, and six prominent horn-like cephalic setae previously unknown in the Dorylaimida. Also, the heavily annulated cuticle divided into plates by numerous longitudinal striae is unique in the dorylaims.

This interesting nematode is described herein as the representative of a new genus, family, and superfamily. The Order Dorylaimida is emended as specified below, so that this nematode could be placed in this major group to which it apparently belongs.

The new generic name, *Encholaimus*, suggests "spear in the throat," referring to the development of the spear in the esophagus as in other dorylaims. It is of the masculine gender and is the latinized combination formed from the Greek "enchos," meaning spear, and "laimos," meaning throat. The specific Latin name "*taurus*" means "bull," and refers here to the presence of horn-like cephalic setae.

### Order Dorylaimida (De Man, 1876) Pearse, 1942 emended

DIAGNOSIS: Nematoda, Adenophorea. Setae usually absent. Cuticle generally smooth; but sometimes heavily annulated, in some cases longitudinal striae forming plate-like cuticle. Esophagus cylindrical, in 1 or 2 parts; when in 2, the posterior part conoid cylindrical or pyriform. Glands uninucleate, and 3, 5, or 7 in number contained within the confines of the esophagus; all gland openings posterior to the nerve ring. Head with 6–18 inner labial papillae and 6, 10, or 14 outer papillae or setae. Amphids cyathiform, pouch-like or tubular. Stoma with mural tooth or teeth; with an axial stylet, or vestigial, or unarmed. Preanal supplements usually present.

### Encholaimoidea, new superfamily

DIAGNOSIS: Dorylaimida, sub-order Dorylaimina.

Head with setae. Stoma armed. Amphids pouch-like, with slit-like apertures. Esophagus composed of a slender anterior part and an expanded posterior portion. Excretory

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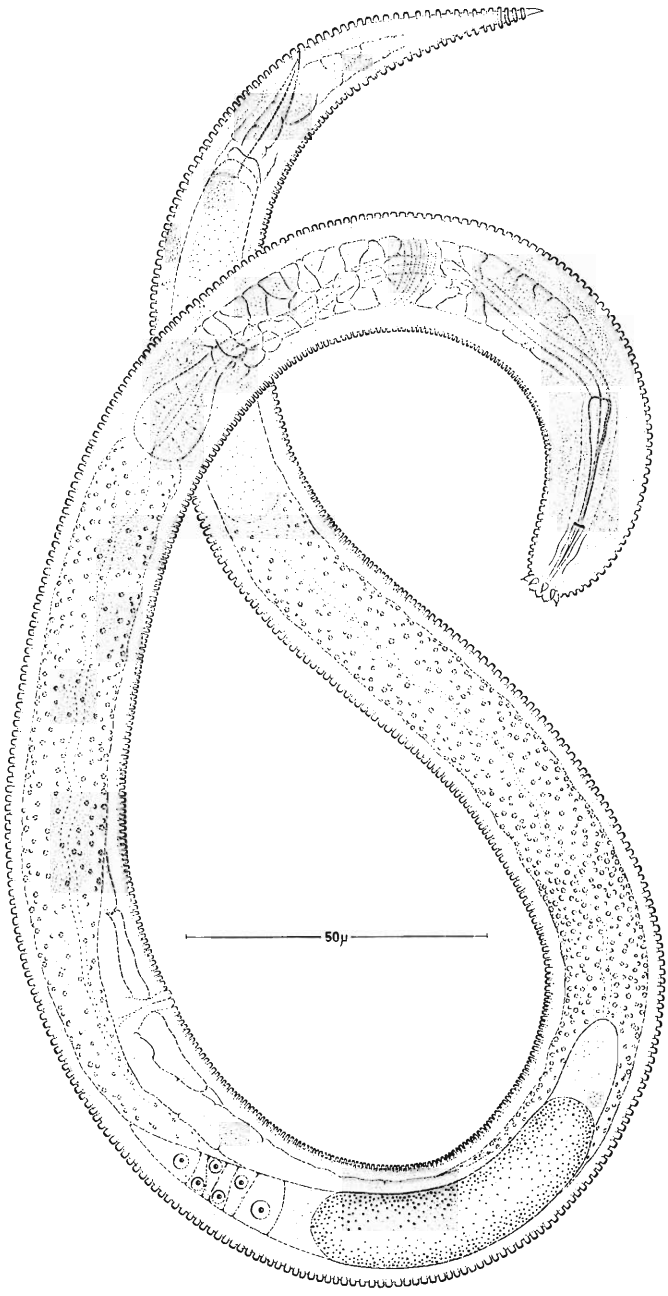


Fig. 1. Full length drawing of female of *Encholaimus taurus*, n. gen., n. sp.

pore absent. No caudal glands. Supplements present.

The presence of cephalic setae is a distinguishing feature of this dorylaimid superfamily.

## Encholaimidae, new family

DIAGNOSIS: Encholaimoidea. Cuticle heavily annulated, often with longitudinal striae giving a plate-like appearance. Stoma armed with an axial spear. Anterior portion of esophagus tubular, posterior third or less enlarged, without a spiral sheath.

The type of cuticle as described, plus the presence of cephalic setae, distinguish this from other families of the Dorylaimida.

TYPE GENUS: *Encholaimus*, n. gen.

## *Encholaimus*, n. gen.

DIAGNOSIS: Encholaimidae. Body of both sexes cylindroid, tapering at extremities. Head with 6 large, horn-like and 4 smaller setae in outer circle. Six labial or perioral papillae in inner circle. Spear with swollen extensions, bearing knobs or flanges. Cuticle heavily annulated, with longitudinal striae forming a plate-like pattern. Lateral pores absent. Ovary 1; vulva a transverse slit. Spicules paired, dorylaimoid in shape.

Distinctive on the basis of the generic characters as specified. No other known genus.

TYPE SPECIES: *Encholaimus taurus*, n. sp.

### *Encholaimus taurus*, n. sp.
(Figs. 1 and 2 A–E)

MEASUREMENTS (15 females): Length 0.55 mm (0.47–0.61); a = 27.1 (24–29); b = 4.8 (4.1–5.5); c = 13.8 (13–15); V = 40% (37–41); spear 18 $\mu$ (17–20); spear extensions 12 $\mu$ (11–12); total 30 $\mu$ (29–31).

HOLOTYPE (female): Length 0.56 mm; a = 27; b = 4.9; c = 14; V = 40%; spear and spear extensions 31 $\mu$; body width 21 $\mu$; tail 39 $\mu$ in length.

DESCRIPTION OF FEMALES: Body tapering anteriorly and more so posteriorly as illustrated (Fig. 1). Body width averaging 20 $\mu$. Head with a prominent outer circle of 10 horn-like cephalic setae, 6 of which are large while 4 are about one-half the size. These surround an inner circle of 6 conspicuous labial papillae on a labial crown (see Fig. 2 A and D especially). Amphid apertures large, slit-like. Cuticle heavily annulated, with longitudinal striae, numbering 24 at mid-body, forming a plate-like appearance. Spear delicate, with distinct guide ring; spear extension swollen posteriorly, with large flanges as illustrated. Esophagus tubular for much of its length, terminating posteriorly in a pyriform, basal bulb measuring about 15% of the esophageal length. Intestine filled with globular granules, details obscure. Ovary 1, dorylaimid, extending posteriorly and then reflexed for almost one-half its length. Vulva a transverse slit, located in anterior half of body. Anus distinct. Tail conical, appearing as in Fig. 1, averaging 39 $\mu$. Lateral pores not detected.

ALLOTYPE (male*): Length 0.56 mm; a = 29; b = 4.5; c = 18; spear 18 $\mu$, spear extension 13; total 31 $\mu$.

Male similar to female in general size and shape. Body width 19 $\mu$. Head, cephalic setae, cuticle, spear and spear extensions, and esophagus also as in female. Testes 2, details obscured. Spicules paired, dorylaimid in shape, measuring 28 $\mu$. Gubernaculum absent. Tail curving ventrally, conical in shape as illustrated in Fig. 2 E, and measuring 31 $\mu$ in length. Two preanal supplements present.

DIAGNOSIS: *Encholaimus* with above measurements and description. No other species known.

HOLOTYPE (female): Collected by Karl Maramorosch November, 1963 and received 20 November 1963. Trinidad. Slide T-83t, United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

ALLOTYPE (male): Same data as holotype. Slide T-84t, United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

PARATYPES (females and larvae): United States Department of Agriculture Nematode Collection, Beltsville, Maryland; and two females, California Nematode Survey Collection, Davis, California.

TYPE HABITAT, HOST AND LOCALITY: Soil around roots of coconut (*Cocos nucifera* L.) at Golden Grove, Trinidad.

DISCUSSION: The nematode described above

* Only one male has been found, and unfortunately it was almost filled with an unidentified sporozoan parasite, obscuring internal details.

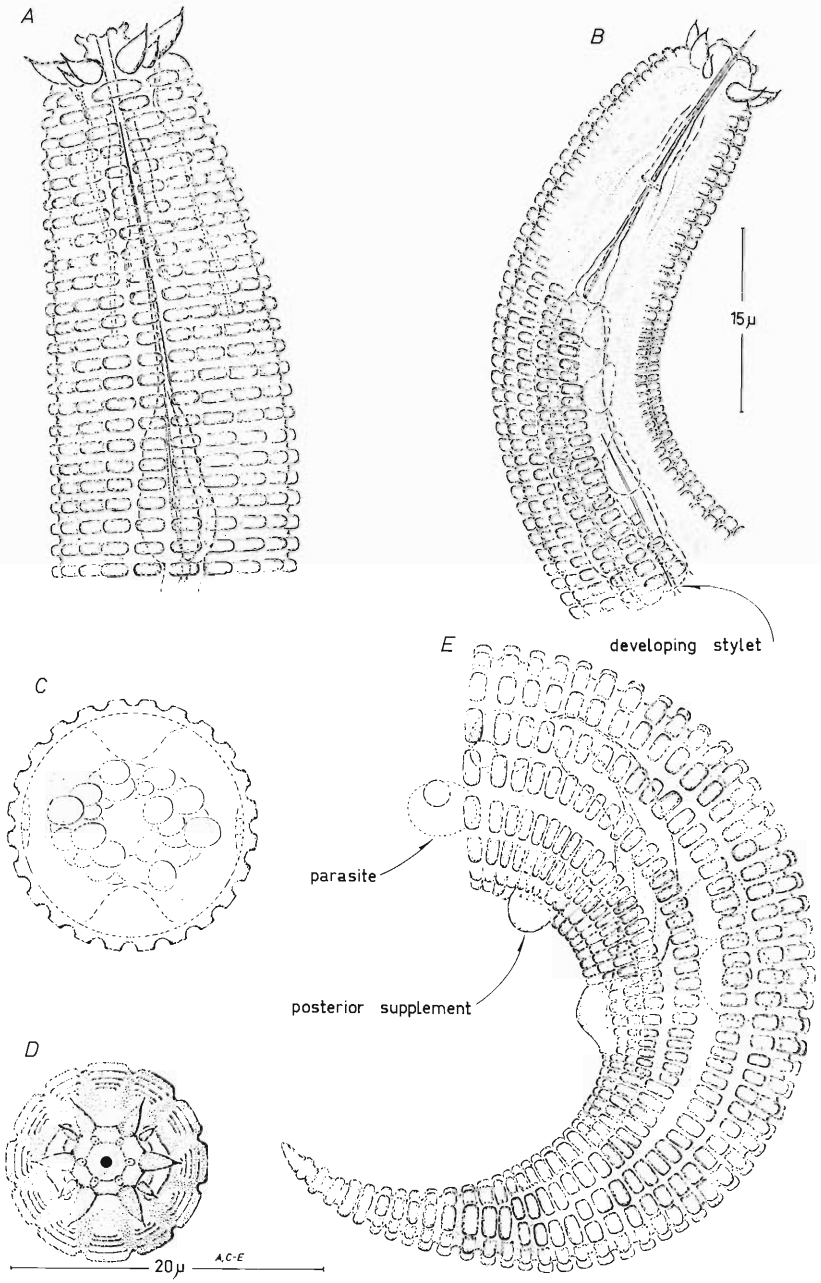


Fig. 2. Drawings of *Encholaimus taurus*, n. gen., n. sp. A, Anterior portion of female. B, Anterior part of immature molting specimen. C, Cross-section of female at mid-body showing, particularly, the cuticle. D, *En face* view of female. E, Posterior portion of male.

obviously has a relationship to other dorylaims, particularly some forms in the Leptonchidae. The esophagus, spear and extensions, reproductive structures, and development of the spear in the esophagus of molting specimens all are characters of the dorylaimid type. On the other hand, the two very outstanding features of this nematode, the prominent cephalic setae and the heavily annulated, plate-like cuticle, have not been previously known in the Dorylaimida. The cephalic setae in particular suggest a relationship to the free-living marine forms of the Enoplida. Further studies on this, and related forms, will be required to establish more clearly the phylogenetic relationships in the dorylaims.

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## Biology of *Mastophorus numidica* (Seurat, 1914) Read and Millemann, 1953 (Nematoda: Spiruridae) with a Description of the Juvenile Stages[1]

WILLIAM G. DYER[2] AND O. WILFORD OLSEN

Life histories of several species of *Mastophorus* have been investigated. Leuckart (1867) and Marchi (1871) showed that *M. muris* (Gmelin, 1790) Chitwood, 1938 develops in meal worms (*Tenebrio molitor*). Adults were obtained in *Mus decumanus* which had eaten infected meal worms. Cram (1926) recovered encysted juveniles from the body cavities of cockroaches (*Blatella germanica*) fed embryonated eggs of *M. muris* (= *Protospirura columbiana* Cram, 1926). Adults were obtained experimentally in rats. Hall (1929) showed that scarabaeid beetles (*Aphodius fimetarius*) are natural intermediate hosts of *M. muris* (= *Protospirura gracilis* Cram, 1924). Marcandier and Pirot (1937) observed juveniles of *M. muris* in the oriental rat flea (*Xenopsylla cheopis*) collected from *Mus decumanus*. Adults were found in the stomach of rats. Miyata (1939) demonstrated that cockroaches (*Periplaneta americana*), fleas (*Leptopsylla musculi, Ceratophyllus anisus, C. fasciatus, X. cheopis*), and moths (*Tinea granella*) could serve as intermediate hosts of *M. muris*.

Brumpt (1931) found that *M. bonnei* (Ortlepp, 1924) Read and Millemann, 1953 from domestic rats develops in cockroaches (*Rhyparobia maderae, Blatella germanica*, and *Periplaneta orientalis*). *M. muricola* (Gedoelst, 1916) Read and Millemann, 1953 was reported from three species of captive monkeys (*Cebus capucinus, Ateles dariensis*, and *Aotus zonalis* by Foster and Johnson (1939). Infective juveniles were identified from the body cavities of cockroaches (*Leucophaea maderae*).

Crook and Grundmann (1964) exposed 15 species of native insects occurring naturally in association with deer mice and four common laboratory insects to eggs of *M. numidica*. Only tenebrionid beetles (*Eleodes tuberculata patruelis*) were naturally and experimentally infected.

Because of the need for more detailed information on the life cycle in *Mastophorus*, further study of *M. numidica* was undertaken. On the basis of the findings, an account of the life cycle and descriptions of the morphology of the developmental stages are presented in this paper.

### MATERIALS AND METHODS

Infective eggs containing first-stage juveniles were obtained as required by immersing ovigerous female worms from the stomachs of *P. maniculatus* in physiological saline. A single

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feeding of eggs was given to individual grasshoppers (*Melanoplus femur-rubrum*), crickets (*Acheta domestica*), and beetles (*Eleodes obsoleta*) which had been starved for at least one day. Specimens of each species were used as controls to determine if natural infections were present. All test and control specimens of *M. femur-rubrum* and *E. obsoleta* were collected in the Cache la Poudre Canyon, Larimer County, northern Colorado. Specimens of *A. domestica* were obtained from Baton Rouge, Louisiana. Anesthetized insects were pinned to the bottom of plastic petri dishes, dissected in 87.6 per cent Ringer's solution, and examined for the presence of juveniles.

Deer mice used for experimental infections were determined to be free of *M. numidica* by frequent examination of the feces over a period of several months. Some were fed infected insects and others isolated cysts containing third-stage juveniles removed from the hemocoels of insects. The feces of these deer mice were checked daily for eggs to determine the prepatent period.

Infected mice were examined by opening the esophagus, stomach, and small intestine separately and agitating each part in a jar of saline to free the worms, the majority of which was found readily upon gross examination. The mucosa was scraped from the walls of each part of the alimentary canal and placed in separate containers of warm saline to allow embedded juveniles to migrate from the tissues into the fluid. The contents of the containers were stirred, sedimented, decanted several times, and examined for juveniles after addition of fresh saline.

Microscopic preparations were made of both living and fixed worms. Since certain structures appeared more clearly in living specimens, some juveniles were relaxed in saline by gently applying heat sufficient to reduce activity. Others were killed in hot saline, fixed in 70 per cent ethanol and 3 per cent glycerine, cleared, dehydrated, and mounted in glycerine. A phase contrast microscope was used in morphological studies.

Drawings of the several stages were prepared from fixed and living specimens. All measurements are in millimeters except where otherwise indicated.

TABLE 1. Results of feeding 40 eggs of *Mastophorus numidica* per insect to grasshoppers (*Melanoplus femur-rubrum*), crickets (*Acheta domestica*), and beetles (*Eleodes obsoleta*); 24 days after exposure.

| Insects | No. examined | No. infected | No. of cysts recovered | |
|---|---|---|---|---|
| | | | Mean | Range |
| *M. femur-rubrum* | | | | |
| Exposed | 50 | 31 | 9.5 | 2–19 |
| Control | 42 | 1 | — | — |
| *A. domestica* | | | | |
| Exposed | 46 | 18 | 8.3 | 6–18 |
| Control | 95 | 0 | — | — |
| *E. obsoleta* | | | | |
| Exposed | 20 | 18 | 8.9 | 1–15 |
| Control | 12 | 4 | 0.5 | 1–2 |

## RESULTS

Experimental infections were obtained in all three species of insects (*M. femur-rubrum*, *A. domestica*, and *E. obsoleta*) fed eggs of *M. numidica* (Table 1). Control specimens of *M. femur-rubrum* and *E. obsoleta* showed natural infections of 2.4 and 33.3 per cent, respectively.

Following is an account of the life cycle of *M. numidica* as it occurs in the cricket (*A. domestica*) and the deer mouse. The verbal descriptions and the illustrations are based on specimens from these hosts.

### *Development and morphology in the cricket*

About 3.5 to 4.5 hr postinfection, the crop contained eggs which were 0.054 to 0.059 by 0.043 to 0.046, embryonated, thick shelled, and otherwise indistinguishable from those occurring in the definitive host's feces. At about 7.5 hr most eggs were in the stomach, a few still in the crop and gizzard; some evidently had hatched, as first-stage juveniles were found in the intestine. At about 10 to 15 hr neither eggs nor juveniles were observed in the digestive tract.

FIRST-STAGE JUVENILE: At 10.5 hr postexposure, the abdominal portion of the hemocoel contained a few first-stage juveniles (Fig. 1). Five days after exposure both free and encysted first-stage juveniles were present, and measured 0.392 to 0.407 by 0.045 to 0.055; anterior end rounded; posterior portion tapering slightly with tip of tail ending in characteristic short conical process; cuticle thin, trans-

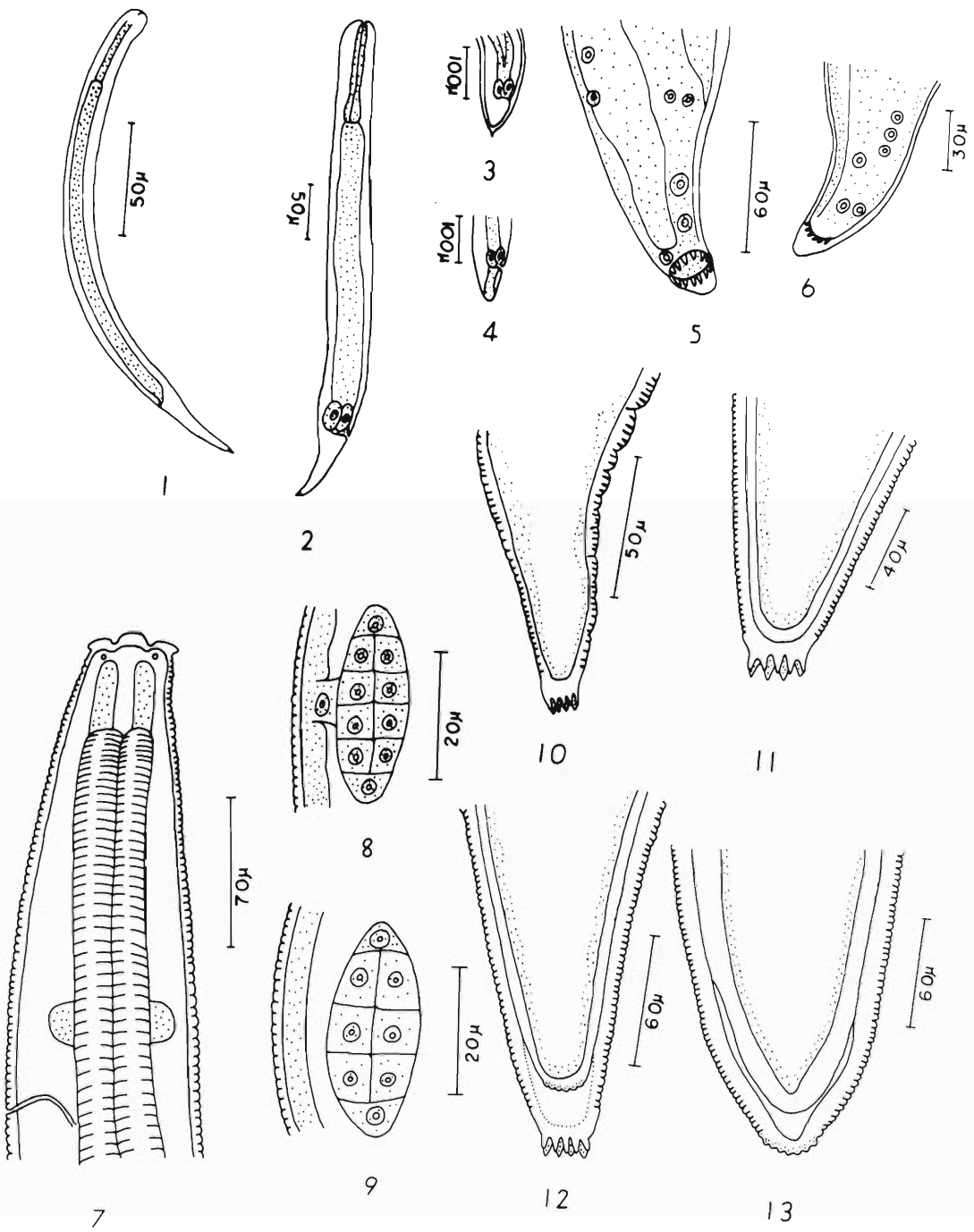

TABLE 2. Age at which third-stage juveniles of *Mastophorus numidica* become infective in cysts in *Acheta domestica*.

| No. of deer mice | Age of cyst (days) | Approximate No. of cysts given/mouse | No. of juveniles recovered/ mouse |
|---|---|---|---|
| 2 | 10 | 35 | 0 |
| 3 | 22 | 20 | 0 |
| 5 | 27 | 20 | 0 |
| 4 | 32 | 20 | 0 |
| 4 | 35 | 30 | 0 |
| 3 | 38 | 30 | 10 |
| 3 | 40 | 30 | 13 |
| 2 | 45 | 35 | 7 |

TABLE 3. Experimental infection of deer mice by feeding each a single cricket exposed to eggs of *Mastophorus numidica*.

| No. of deer mice | No. of eggs fed to crickets | Time after exposure (days) | No. of juveniles recovered/ mouse |
|---|---|---|---|
| 2 | 40 | 10 | 0 |
| 3 | 40 | 22 | 0 |
| 3 | 40 | 27 | 0 |
| 4 | 50 | 32 | 0 |
| 3 | 50 | 35 | 0 |
| 3 | 50 | 38 | 12 |
| 3 | 50 | 40 | 15 |
| 2 | 50 | 45 | 13 |

parent, with very fine transverse striations; oral opening leading into a transparent esophagus approximately one-fifth of body, slightly swollen at posterior end; intestine granular, connecting posteriorly with a very short rectum surrounded by two rectal glands (Fig. 2).

Juveniles undergo the first molt 7 to 9 days after experimental infection as indicated by a detached cuticle in the tail region (Fig. 3). On the 8th day, some showed a partially detached cuticle, others had completed the first molt and were in the second stage.

SECOND-STAGE JUVENILE: Only second-stage juveniles were observed by the ninth day. The bulbous swelling at the posterior part of the esophagus was not as prominent as in the preceding stage. Fourteen-day-old juveniles measured 2.068 to 2.189 by 0.77 to 0.81; body tapering toward each extremity; tip of tail rounded, having lost the conical process with shedding of first cuticle (Fig. 4); cuticle thin, transparent and with very fine transverse striations; oral opening leading into a buccal capsule 0.001 to 0.012 deep; esophagus about one-third of body length, divided into muscular and glandular portions, terminal swelling absent; intestine simple and narrow; rectum surrounded by three large rectal glands; nerve ring and excretory pore 0.108 to 0.111 and 0.130 to 0.135 from anterior extremity, respectively.

Juveniles recovered on the 19th day post-infection were molting. The partially detached cuticle was observed at the posterior end (Figs. 5, 6). By the 21st day, a few juveniles still showed a partially detached cuticle while the majority had completed the second molt and were in the third stage of development.

THIRD-STAGE JUVENILE: By the 22nd day, third-stage juveniles only were observed. Those recovered on the 24th day measured 2.684 to 2.737 by 0.094 to 0.099; body tapering toward each extremity; tail conical terminating in a characteristic rosette of spinous processes (Fig. 10); cuticle thick and transversely striated; lips trilobed similar to adult, median lobe larger than dorsal or ventral (Fig. 7), each lobe armed with two teeth; four submedian papillae present, located at bases of dorsal and ventral lobes; buccal capsule 0.040 to 0.042 deep; esophagus similar to that of second-stage juvenile but broader near posterior end; nerve ring and excretory pore 0.125 to 0.128 and 0.145 to 0.148 from anterior end, respectively. Sex could be differentiated at this stage; male genital primordium elliptical in shape about 0.032 to 0.034 by 0.015, located ventrally between body wall and intestine, 0.630 to

←

Figs. 1–13. Various stages in the development of *Mastophorus numidica*, drawn with the aid of camera lucida. 1–2. First-stage juvenile, complete worm. 3. First-stage juvenile, undergoing first molt, caudal end. 4. Second-stage juvenile, posterior extremity, lateral aspect. 5–6. Second-stage juvenile, undergoing second molt, lateral aspect of caudal end. 7. Third-stage juvenile, anterior extremity, lateral aspect. 8. Genital primordium attached to body wall, third-stage juvenile female, lateral view. 9. Genital primordium of third-stage juvenile male, lateral view. 10. Third-stage juvenile, posterior extremity, lateral aspect. 11. Third-stage juvenile, prior to third molt, lateral aspect of caudal end. 12. Third-stage juvenile, undergoing third molt, lateral aspect of caudal end. 13. Fourth-stage juvenile, undergoing fourth molt, dorsal aspect of caudal end.

TABLE 4. Determination of the prepatent period of *Mastophorus numidica* in the deer mouse.

| No. of deer mice | No. of encysted juveniles administered/ mouse | Age of cysts from crickets (days) | Ova first appeared in feces (days) |
|---|---|---|---|
| 1 | 25 | 10 | * |
| 1 | 25 | 22 | * |
| 1 | 23 | 27 | * |
| 2 | 24 | 32 | * |
| 4 | 25 | 35 | * |
| 3 | 22 | 38 | 35 |
| | | | 35 |
| | | | 41 |
| 4 | 22 | 40 | 37 |
| | | | 40 |
| | | | 42 |
| | | | 42 |
| 2 | 22 | 45 | 35 |
| | | | 37 |

* Ova absent in one daily examination.

0.635 from posterior end, composed of 2 large epithelial cells enclosing a group of germinal cells (Fig. 9); female genital primordium also somewhat elliptical, about 0.031 by 0.011, attached to body wall ventrally by means of a cell, 0.874 to 0.876 from tip of tail (Fig. 8).

### *Development and morphology of worms in the deer mouse*

Infection was established in deer mice fed 38- to 45-day-old encysted juveniles removed from the hemocoels of crickets (Table 2) as well as in those fed crickets 38 to 45 days after exposure of the latter to eggs of *M. numidica* (Table 3). Similar results were obtained with grasshoppers and beetles. Since older juveniles were not administered, it was not determined if infectivity of juveniles decreases with age.

Transition to the fourth stage occurred in the stomach of the deer mouse 8 to 11 days after ingestion of cysts containing infective third-stage juveniles. In a late phase of the impending molt, two thick cuticles are clearly observable, an outer one with a rosette of spinous processes at the terminus of the tail and an inner one with small elevations at the caudal tip (Figs. 11, 12). By the end of the 11th day, all juveniles examined had completed the third molt and were in the fourth stage.

FOURTH-STAGE JUVENILE: Examination of a deer mouse 14 days after ingestion of cysts containing third-stage juveniles revealed fourth-stage females only; body 7.425 to 8.360 by 0.187 to 0.196; cuticle thick and transversely striated; lips well developed; buccal capsule 0.072 to 0.080 deep; nerve ring and excretory pore 0.180 to 0.192 and 0.252 to 0.268 from anterior end, respectively; primitive vulva posterior to mid-body; vagina short and curved posteriorly; muscular ovijector followed by short trunk which branches into an anterior and a posterior uterus; oviduct narrow; tail conoid with small cuticular elevations at tip giving it a rough appearance.

Two females recovered from the stomach of a deer mouse examined 15 days postinfection were beginning the fourth molt. They showed a partially detached thick outer cuticle with cuticular elevations at the caudal tip and the thick inner cuticle was smooth at the tip of the tail (Fig. 13). By the 17th day, all specimens examined had completed the fourth molt and were fifth stage. The adult stage has been described by Seurat (1914) and warrants no further description here.

### *Determination of prepatent period*

At 35 to 42 days after ingestion of infective juveniles, sexually mature adults were present as evidenced by the appearance of eggs in the feces of experimentally infected deer mice (Table 4).

## DISCUSSION

With the exception of studies reported by Hall (1929) and Crook and Grundmann (1964), all prior life history investigations of *Mastophorus* were conducted with insects which are commonly used in the laboratory and probably are not the intermediate hosts under natural conditions. Though several orders of insects (Coleoptera, Orthoptera, Siphonaptera, and Lepidoptera) have been found to contain members which serve as intermediate hosts of *Mastophorus,* grasshoppers have not been reported previously to function as such. Results with other orthopteran insects suggest that grasshoppers may play an important role under natural conditions as intermediate hosts for species of *Mastophorus* other than *M. numidica.*

Crook and Grundmann (1964) reported that grasshoppers (*M. femur-rubrum*) did not be-

come infected when exposed to feces of deer mice containing eggs of *M. numidica*. However, in the present experiment, grasshoppers became infected when fed the eggs concentrated on a small piece of lettuce which had been thoroughly washed with tap water. Since grasshoppers are not coprophagic, it seems probable that they become infected in nature by eating contaminated vegetation rather than fecal matter.

The time intervals required in the intermediate hosts for development to infectivity by juveniles of *M. muris* and *M. numidica* are similar. Cram (1926) found that 41 days were necessary for development of infective *M. muris* in the hemocoel of *Blatella germanica*. Crook and Grundmann (1964) reported that infective cysts occurred in the hemocoel of *E. tuberculata patruelis* 40 days after ingestion of eggs of *M. numidica*. Similar results were obtained in the present study; 38 days after ingestion of eggs of *M. numidica* by the grasshopper (*M. femur-rubrum*), infective cysts were observed in the hemocoel. The prepatent period reported for *M. muris* is about 115 days as compared with about 36 days for *M. numidica*, as found by Crook and Grundmann (1964). In the present study, this period ranged from 35 to 42 days.

## SUMMARY

An account of the life cycle of *Mastophorus numidica* as it occurs in the cricket (*Acheta domestica*) and the deer mouse (*Peromyscus maniculatus*) is described and illustrated. Experimental infections were obtained in all three species of insects (*Melanoplus femur-rubrum*, *Eleodes obsoleta*, and *A. domestica*) fed eggs of *M. numidica*. Control specimens of *M. femur-rubrum* and *E. obsoleta* showed natural infections. The first and second molts occur in the hemocoel of crickets 7 to 9 and 19 to 22 days postinfection, respectively.

Infection was established in deer mice fed 38- to 45-day-old encysted juveniles removed from the hemocoel of crickets as well as in those fed crickets 38 to 45 days after exposure of the latter eggs of *M. numidica*. Similar results were obtained with grasshoppers and beetles. The fourth and fifth molts occur in the stomach of deer mice 8 to 11 and 15 to 17 days postinfection, respectively. The prepatent period ranged from 35 to 42 days.

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# Annotated Record of Some Previously Described Digenetic Trematodes of Amphibians and Reptiles from the Philippines, Korea, and Matsu Island[1]

JACOB H. FISCHTHAL AND ROBERT E. KUNTZ[2]

The trematodes of this report were part of a collection made by the junior author while a member of the U. S. Naval Medical Research Unit No. 2, Taipei, Taiwan. While this report contains no new forms we are presenting the data in order to supplement previous body and organ measurements so that worms of different size ranges, which may represent differences due to allometric growth, are not described as new species; to note intraspecific variations so that such variants are not described as new species; to detail anatomical descriptions which have been lacking or which have been previously presented without such detail; to gather additional data for clarifying statements in the literature which may be questionable; to indicate new and probable synonymies; and to note new host and geographic distribution records. Parasites were washed in saline, killed in hot water, and transferred immediately to FAA fixative. After 4–8 hours they were stored in 70% alcohol plus 2% glycerine. All measurements are in microns.

## FAMILY PARAMPHISTOMIDAE

### *Diplodiscus amphichrus* Tubangui, 1933

SYNONYM: *Diplodiscus sinicus* Li, 1937.

HOST: *Rana limnocharis vittigera* (Günther) (syn. *R. vittigera* Wiegmann) (Ranidae).

HABITAT: Small intestine.

LOCALITY: Manila, Luzon Island, Philippines.

DATES: 11, 13 December 1961.

SPECIMENS: USNM Helm. Coll. No. 61700 (three slides with one adult specimen each).

MEASUREMENTS AND SOME PERTINENT DATA (based on eight adults and 40 immature worms from nine hosts; one mature and two young adults measured): Body 1,645 to 2,475 by 270 to 780; preoral body 0 to 16; pigment granules of disintegrated cercarial eyespots concentrated from just postbifurcal to posterior half of oral sucker but may extend beyond these levels; oral sucker 136 to 250 by 111 to 138; esophagus 189 to 295 long; acetabulum 370 to 642 by 410 to 575; testis 157 to 240 by 121 to 295; cirrus sac 60 to 110 by 48 to 110; ovary 77 to 194 by 61 to 194; vitellaria confluent anteriorly and usually posteriorly, interrupted at testis level, 21 to 25 follicles anterior to interruption and 16 to 19 posterior; cecal bifurcation to genital pore 205 to 420, to testis 370 to 420, to ovary 595 to 835; nine eggs 96 to 107 by 66 to 80.

DISCUSSION: This species was originally described by Tubangui (1933) from *Rana* spp. from the same island as the present specimens; later, Tubangui (1947) specifically identified *Rana vittigera* as the only host. Li (1937a) described a new species, *D. sinicus*, from the same host species as our specimens from China and from *R. regulosa* Wiegmann from China and Amoy Island. Bravo (1941) declared Li's species a synonym of *D. amphichrus*. Our nine hosts harbored one (mature adult), two (two mature adults in one, two immature in another), three (one young adult and two immature in one, three immature in each of two others), seven (immature), nine (three young adults and six immature), and 18 (one mature adult and 17 immature) worms, respectively.

## FAMILY PLEUROGENIDAE

### *Pleurogenoides taylori* (Tubangui, 1928) Travassos, 1930

SYNONYMS: *Pleurogenes taylori* Tubangui, 1928; *Pleurogenoides hashimi* Rohde, 1963.

HOST: *Rana limnocharis vittigera* (syn. *R. vittigera*) (Ranidae).

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TABLE 1. *Pleurogenoides taylori.* Measurements of eight specimens in microns.

| Specimen No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Body | 284×133 | 286×121 | 302×143 | 370×174 | 495×265 | 506×318 | 705×475 | 980×501 |
| Forebody | 145 | 160 | 167 | — | 290 | 264 | 276 | 342 |
| Hindbody | 82 | 70 | 77 | — | 108 | 133 | 245 | 285 |
| Oral sucker | 59×61 | 69×69 | 67×68 | 73×78 | 97×102 | 85×99 | 138×153 | 133×155 |
| Acetabulum | 57×57 | 56×52 | 58×56 | — | 97×97 | 109×90 | 132×132 | 136×143 |
| Pharynx | 28×34 | 34×33 | 28×34 | 31×42 | 51×49 | 48×57 | — | 76×57 |
| Right testis | 85×46 | 55×44 | 77×60 | 99×73 | 85×73 | 169×148 | 145×138 | 157×172 |
| Left testis | 93×40 | 53×46 | 77×59 | 94×51 | 126×70 | 128×108 | 145×134 | 184×111 |
| Cirrus sac | 160×59 | 183×34 | 131×44 | 150×46 | 281×85 | 283×70 | 290×136 | 345×159 |
| Ovary | — | 46×46 | — | 44×34 | 46×38 | 85×85 | 99×119 | 133×99 |
| Seminal receptacle | — | — | — | — | — | — | 77×97 | 65×80 |
| Eggs | — | — | — | — | — | 30–31×15–16 | 28–32×15 | 30–32×15–18 |

HABITATS: Stomach, small intestine.

LOCALITY: Manila, Luzon Island, Philippines.

DATE: 13 December 1961.

SPECIMENS: USNM Helm. Coll. No. 61701 (three slides with one adult worm each).

MEASUREMENTS AND SOME PERTINENT DATA (based on two mature adults from one host, and two young adults and six immature from a second): Measurements for all but two immature specimens presented in Table 1. Sucker length ratio 1:0.81 to 1.28; in four adults preoral body 3 to 10 long and vitelline follicles 14 to 16 in number.

DISCUSSION: In Table 1 specimens 1 to 4 are immature, 5 has vitelline cells and shell material in the uterus but no eggs, 6 is a young adult with eggs, and 7 and 8 are mature adults. This species was described by Tubangui (1928) from the intestine of *Rana vittigera* from Luzon and redescribed by Li (1937a) from *R. regulosa* from China.

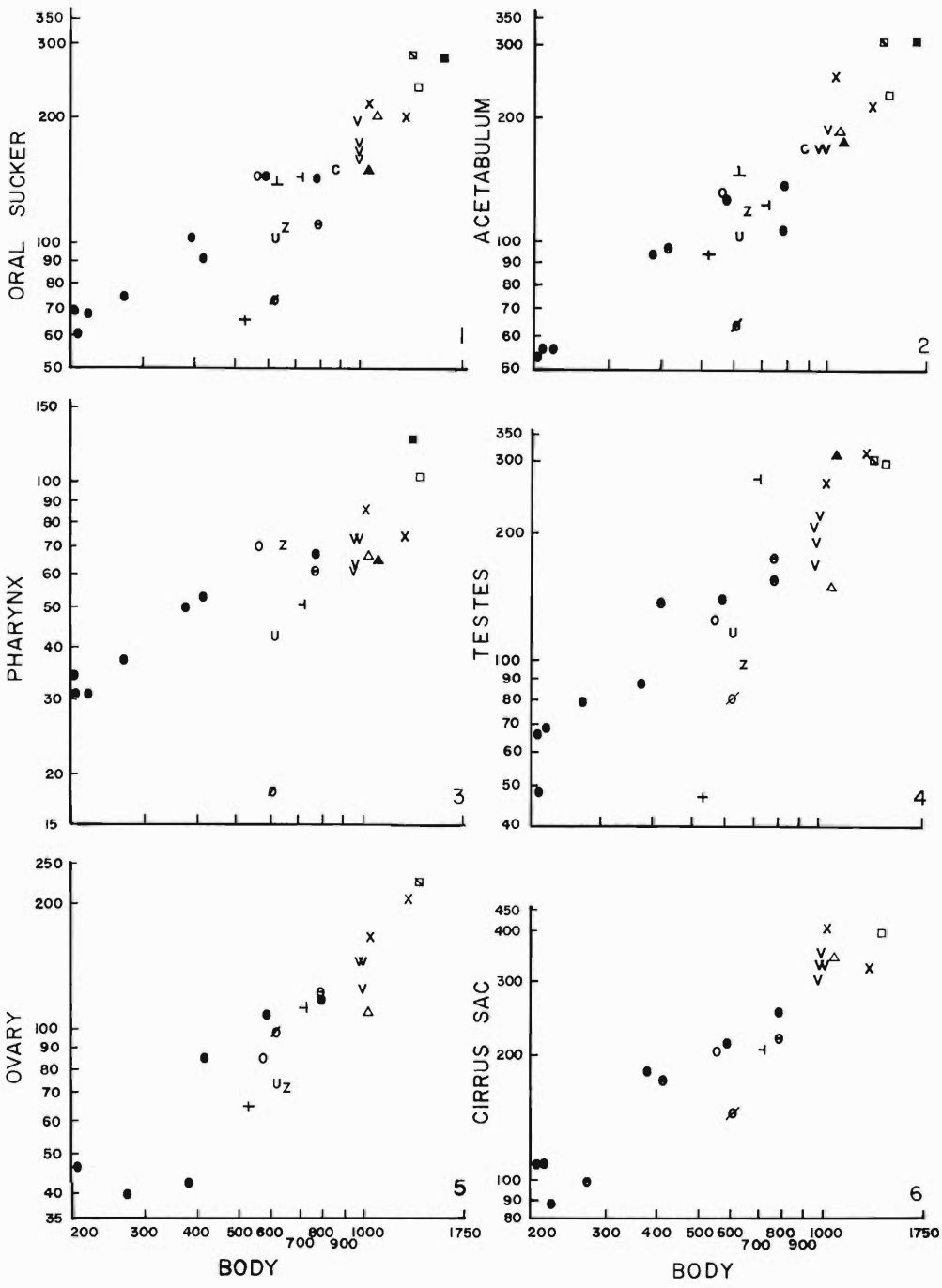
We declare *P. hashimi,* described by Rohde (1963) from *Rana cancrivora* Gravenhorst from Malaya, a synonym of *P. taylori.* Rohde, without considering Li's description of *P. taylori,* stated that his form appears closest to the latter species but differs in its larger size, distinctly larger testes and ovary, and smaller eggs (28 to 33 by 11 to 13). The latter point is invalid as Tubangui gave the egg size as 31 to 33 by 15 to 18 and Li as 27.3 to 31.2 by 11.7 to 15.6. There is an overlap in gonad size with Li's specimens. The relative differences in body and gonad sizes may be due to allometric growth as all described specimens of *P. taylori,* as well as ours, are smaller than those of *P. hashimi.*

Huxley (1932) expressed allometric growth by the formula $y = bx^k$, where $y$ = organ size, $x$ = body size, $k$ = allometric exponent, and $b$ = constant. He converted this formula into $\log y = \log b + k \log x$ which corresponds to a straight line when shown graphically in a double-logarithmic system of coordinates. Rohde (1966) applied this method in determining species synonymy in the genus *Anchitrema* Looss, 1899 (Lecithodendriidae). We have followed Rohde's methods in attempting to show further that *P. hashimi* is a synonym of *P. taylori.* Our graphs substantiated our opinion. However, to ascertain whether we were justified in interpreting the graphs as we did we similarly plotted on the same graphs most of the other species of *Pleurogenoides* Travassos, 1921 (Figs. 1 to 6). The graphs show interestingly that most species of the genus, with the exception of *P. bufonis* Kaw, 1943 and *P. minus* (Pigelevsky, 1931), exhibit basic similarities in allometric growth of the body and its organs. We can not conclude that all but the two exceptions are synonymous with *P. taylori*; for example, *P. solus* (S. J. Johnston, 1912) has significantly smaller eggs (20 microns long) and the number, size, and distribution of the vitelline follicles are quite different for *P. compactus* Shtrom, 1940 and *P. gastroporus* (Lühe, 1901). It may be that several of the remaining species are synonymous but further study is necessary. There is a danger in overemphasizing allometric growth in determining species synonymy since many species within any single genus may show much similarity. Fortunately, Rohde (1966) had in his collection specimens of *Anchitrema* covering the size ranges of the species he placed in synonymy.

### FAMILY PLAGIORCHIIDAE

### *Glypthelmins staffordi* Tubangui, 1928

HOST: *Rana limnocharis vittigera* (syn. *R. vittigera*) (Ranidae).



HABITATS: Stomach, small intestine.

LOCALITY: Manila, Luzon Island, Philippines.

DATES: 11, 13 December 1961.

SPECIMENS: USNM Helm. Coll. No. 61702 (four slides with one adult each).

MEASUREMENTS AND SOME PERTINENT DATA (based on 24 adult and 20 immature worms, eight adults measured): Body 1,454 to 1,960 by 397 to 625; forebody 465 to 645, hindbody 780 to 1,286, preoral body 12 to 39, posttesticular space 615 to 1,015, postvitellarian space 320 to 475, postcecal space 120 to 440; oral sucker 151 to 206 by 152 to 222; acetabulum 80 to 125 by 96 to 130, center at level of anterior one-third to four-tenths of body length; sucker length ratio 1:0.48 to 0.67; prepharynx 15 to 39 long; pharynx 75 to 111 by 75 to 121, transversely or longitudinally elongate; esophagus 10 to 85 long; cecal bifurcation 111 to 184 preacetabular; testes round to longitudinally or transversely elongate; left testis 157 to 206 by 160 to 206, usually more anterior than right testis, overlapping acetabulum as much as 105 to lying entirely postacetabular as much as 44; right testis 162 to 215 by 114 to 189, overlapping acetabulum as much as 57 to lying entirely postacetabular as much as 92; cirrus sac 99 to 182 (longitudinal extent) by 63 to 87, wall moderately thick, muscular, anteriormost margin 41 to 92 preacetabular; ovary 111 to 143 by 114 to 186, smaller than testes but occasionally may be slightly wider, round to longitudinally or transversely elongate; vitellaria may commence at level about halfway between cecal bifurcation and acetabulum, that is, slightly anterior to anteriormost margin of cirrus sac; 32 operculate eggs 29 to 37 by 19 to 27.

DISCUSSION: The eight infected hosts harbored one (adult), two (adult, in two), three (adult), four (one adult and three immature in one, four immature in another), seven (four adult and three immature), and 21 (11 adult and 10 immature) worms, respectively. This species was described by Tubangui (1928) from *Rana vittigera* from Luzon, and redescribed by Li (1937a, b) from *Rana regulosa* Wiegmann from China, by Yamaguti and Mitunaga (1943) from *Bufo melanostictus* Schneider (Bufonidae) from Formosa and by Yuen (1962) from *Rana macrodon* Kuhl, *R. erythraea* (Schlegel) and *R. cancrivora* Gravenhorst from Singapore. Nasir (1966) briefly reviewed the genus.

*Styphlodora renalis* Tubangui, 1933

HOST: *Naja naja* (L.) (Elapidae).

HABITATS: Kidney, small intestine.

LOCALITY: Zamboanga, Mindanao Island, Philippines.

DATES: 23 January, 16 February 1961.

SPECIMENS: USNM Helm. Coll. No. 61703 (eight slides with two specimens each from kidney and one slide with one worm from small intestine).

MEASUREMENTS AND SOME PERTINENT DATA (based on 63 adults from kidney of one cobra, 11 measured): Body 1,657 to 2,827 by 560 to 1,210; forebody 445 to 550, hindbody 965 to 2,000, posttesticular space 472 to 1,080, postcecal space 200 to 570; suckers round to longitudinally or transversely elongate; oral sucker 157 to 227 by 167 to 230; acetabulum

←

EXPLANATION OF GRAPHS

Key to symbols in Figures 1–6:

○ *Pleurogenoides taylori* (Tubangui, 1928); average data.
⊖ *P. taylori*, after Li, 1937; average data.
● *P. taylori*, our specimens; individual data.
⌀ *P. bufonis* Kaw, 1943; average data.
△ *P. compactus* Shtrom, 1940; average data.
▲ *P. freycineti* (S. J. Johnston, 1912); average data.
⧅ *P. gastroporus* (Lühe, 1901); average data.
■ *P. gastroporus*, after Travassos, 1930; average data.
□ *P. gastroporus*, after Simha, 1958; average data.
V *P. hashimi* Rohde, 1963; individual data.
× *P. ifranensis* Dollfus, 1958; individual data.
U *P. japonicus* (Yamaguti, 1936); average data.
+ *P. minus* (Pigelevsky, 1931); average data.
⊣ *P. sitapurii* (Srivastava, 1934); average data.
⊥ *P. sphaericus* (Klein, 1905); average data.
Z *P. solus* (S. J. Johnston, 1912); average data.
C *P. tener* (Looss, 1898), after Travassos, 1930; average data.

The size of the specimens of *Pleurogenoides* and their organs is given as average diameter calculated from two dimensional measurements: (length + width)/2.

215 to 325 by 220 to 360, center at level of approximate anterior one-fourth to one-third of body length; sucker length ratio 1:1.11 to 1.47; prepharynx 17 to 49 long; pharynx 97 to 136 by 84 to 114, longer than wide by 9 to 31; esophagus 24 to 63 long; cecal bifurcation close to acetabulum or well separated, 48 to 182 preacetabular; testes round to longitudinally or transversely elongate, smooth to slightly irregular or somewhat lobed, usually relatively narrowly separated from one another by ascending uterus but may be widely separated or in contact, usually diagonal in position with left testis anterior to right but almost symmetrical in some; anterior testis 197 to 330 by 177 to 350, 35 to 275 postacetabular; posterior testis 206 to 330 by 186 to 400, 197 to 625 postacetabular; cirrus sac 265 to 605 (longitudinal extent) by 90 to 152, very thick walled, muscular, proximal portion may be straight or bent sharply in any direction, usually commencing postacetabular (up to 175) but may begin dorsal to latter's posterior one-fourth or as in one specimen anterior to its midlevel (lying obliquely at its anterodextral margin with proximal portion overlapping right cecum dorsally), anteriormost extent 48 to 110 preacetabular; cirrus sac containing long, relatively thin walled, tubular to saccular, occasionally bipartite seminal vesicle, short pars prostatica, prostate cells, and long, very thick, muscular, protrusible cirrus; genital pore on midline or to its left at any level between cecal bifurcation and acetabulum; ovary smooth, 115 to 212 by 114 to 177, up to 115 postacetabular or may overlap as much as posterior one-seventh of latter; seminal receptacle 77 to 168 by 85 to 169, usually smaller than ovary but may be same size or slightly larger, usually posterodorsal to ovary but may be entirely dorsal or posterior; metraterm thick walled, muscular, relatively straight, surrounded by gland cells, commencing dorsal to approximate midlevel of acetabulum, ascending left of cirrus sac, usually extending slightly anterior to latter before curving posteroventral to open into genital atrium; vitelline follicles usually large but may be relatively small, 8 to 22 in each field; fields narrow to broad in width, commencing at level of posterior two-thirds of acetabulum, terminating at level of body of anterior testis but may end at level of its anterior margin; right vitelline field 255 to 720 long, left 189 to 515; right field extending 155 to 540 postacetabular, left 132 to 485; 42 operculate eggs 36 to 51 by 18 to 25.

MEASUREMENTS AND SOME PERTINENT DATA (based on one adult from small intestine of second cobra): Body 2,812 by 595; forebody 627, hindbody 1,950, posttesticular space 1,100, postcecal space 177; oral sucker 196 by 193; acetabulum 235 by 230, center at level of anterior one-fourth of body length; sucker length ratio 1:1.19; prepharynx 52 long; pharynx 120 by 128; esophagus 74 long; cecal bifurcation well separated from acetabulum, 177 preacetabular; testes smooth; anterior testis 260 by 230, 342 postacetabular; posterior testis 395 by 240, 455 postacetabular; cirrus sac 475 (longitudinal extent) by 113, commencing 134 postacetabular, anteriormost extent 111 preacetabular; seminal vesicle long, tubular, bipartite; genital pore slightly closer to acetabulum than cecal bifurcation; ovary 165 by 140, 95 postacetabular; seminal receptacle 186 by 167, posterodorsal to and larger than ovary; right vitelline field 410 long, left 435; right field extending 325 postacetabular, left 315; five eggs 39 to 41 by 20 to 22.

DISCUSSION: Considerable individual variations occurred as noted for the population of 63 worms from the kidney of a single cobra. Depending upon the combination of characteristics they keyed to *S. serrata* Looss, 1899, or *S. nicolli* Bhalerao, 1936, in the key given by Bhalerao (1936), to the latter two, *S. lachesidis* MacCallum, 1921, or *S. renalis* in the keys by Byrd, Parker, and Reiber (1940) and by Skrjabin and Antipin (1961), to *S. serrata*, *S. nicolli*, or *S. renalis* in the key by Dawes (1941), and to the latter three species or *S. simplexa* (Byrd, Parker, and Reiber, 1940) Dawes, 1942, in the key by Dawes (1942). In all keys the single worm from the small intestine of the second host keyed to *S. najae* described by Nicoll (1912) from the ureters of *Naja naja* (syn. *N. tripudians* Merr.) from India. The 63 specimens appear closest to *S. renalis* described by Tubangui (1933) from the kidney of *Python reticulatus* (Schneider) (Boidae) from Luzon, and to *S. nicolli* described by Bhalerao (1936) from the intestine of *Ptyas mucosus* (L.) (syn. *Zamenis m.* L.) (Colubridae) from India. Our specimens differ

from S. *nicolli* in having fewer and much larger vitelline follicles, in not being constricted at the acetabular level, and in possessing a much wider body. They differ from S. *renalis* in having its acetabulum farther posteriorly and in possessing a prepharynx; no doubt a restudy of the latter species will show a very short prepharynx present. We believe our specimens to be S. *renalis*, including the one from the small intestine; the latter appears different to some extent possibly because of its development in the intestine rather than the kidney. As Nicoll (1912) noted for S. *najae* his specimens, "although mature, are possibly not fully grown." When fully grown they may very well resemble S. *renalis*. In other words, the latter may be a synonym of S. *najae*, but this can not be ascertained at present due to a lack of comparative materials and knowledge of their life cycles.

FAMILY ENCYCLOMETRIDAE

*Encyclometra colubrimurorum* (Rudolphi, 1819) Dollfus, 1929

HOSTS: *Enhydris plumbea* (Boie), *Elaphe rufodorsata* (Cantor) (Colubridae).

HABITAT: Small intestine.

LOCALITIES: San-lun Village, Matsu Island (*E. plumbea*); 12 miles southwest of Seoul, Korea (*E. rufodorsata*).

DATES: 1 August (*E. plumbea*), 17 October (*E. rufodorsata*) 1961.

SPECIMENS: USNM Helm. Coll. No. 61704 (three slides with one specimen each from *E. plumbea*); No. 61705 (one slide with one specimen from *E. rufodorsata*).

MEASUREMENTS AND SOME PERTINENT DATA (based on eight adult and two immature specimens from *E. plumbea*, six adults measured; one adult from *E. rufodorsata*, measured): Body 1,955 to 5,460 by 575 to 1,375; forebody 450 to 1,875, hindbody 1,217 to 3,050, preoral body 39 to 56, posttesticular space 735 to 1,540; oral sucker 255 to 450 by 300 to 502; acetabulum 315 to 535 by 335 to 585, center at level of anterior three- to four-tenths of body length; sucker length ratio 1:1.08 to 1.24; pharynx 157 to 252 by 121 to 295; short prepharynx and esophagus present; cecal bifurcation 60 to 425 preacetabular; ceca subequal in length in nine specimens, equal in two, right usually shorter, posterior extremity to right cecum 390 to 680, to left cecum 140 to 550; testes tandem to diagonal, smooth to slightly lobed; anterior testis 157 to 270 by 186 to 405, overlapping acetabulum 15 in one and 145 to 680 postacetabular in other six; posterior testis 182 to 335 by 180 to 405, 140 to 1,175 postacetabular; cirrus sac 225 to 635 (longitudinal extent) by 73 to 135, entirely preacetabular to considerably overlapping latter, nearly straight to much recurved; ovary 123 to 235 by 123 to 260, usually partly dorsal to acetabulum but may be entirely dorsal or postacetabular; right vitelline field commencing 115 anterior to posterior margin of acetabulum or up to 245 postacetabular, left commencing 142 anterior or up to 395 postacetabular; lateral vitelline fields separate posteriorly or may be confluent; posterior extremity to right vitelline field 110 to 325, to left field 95 to 180; 13 eggs 75 to 94 by 34 to 51.

DISCUSSION: Yeh (1958) reviewed the genus *Encyclometra* Baylis and Cannon, 1924, recognizing three species: *E. colubrimurorum*, *E. japonica* Yoshida and Ozaki, 1929, and *E. asymmetrica* Wallace, 1936. Dollfus (1963) recognized only two of these, declaring the second a synonym of the first. The recovery of *E. colubrimurorum* from Matsu Island, located off the coast of mainland China near Amoy, represents a new geographic distribution record; *Elaphe rufodorsata* is a new host species. It has been reported previously in *Enhydris plumbea* from North Borneo by us (1965) and from Formosa (as *Encyclometra microrchis* Yamaguti, 1933).

FAMILY HARMOTREMATIDAE

*Harmotrema eugari* Tubangui and Masiluñgan, 1936

HOST: *Cerberus rhynchops* (Schneider) (Colubridae, syn. Homalopsidae).

HABITAT: Small intestine.

LOCALITY: Zamboanga, Mindanao Island, Philippines.

DATE: 21 December 1961.

SPECIMENS: USNM Helm. Coll. No. 61706 (three slides with one specimen each).

MEASUREMENTS AND SOME PERTINENT DATA (based on two complete specimens and one with part of preacetabular body missing, from one host): Body 2,266 to 3,171 by 565 to 630;

forebody 420 to 930, hindbody 1,735 to 2,125, posttesticular space 595 to 790, postcecal space 245 to 285, postvitellarian space 195 to 215; oral sucker 90 to 97 by 73 to 85; acetabulum 111 to 116 by 88 to 118, center at level of anterior one-fourth to one-third of body length; sucker length ratio 1:1.20 to 1.23; pharynx 80 by 73 to 77; esophagus 22 to 80 long; testes 109 to 222 apart, longitudinally or transversely elongate, smooth to slightly irregular in outline; anterior testis 145 to 240 by 184 to 242, 500 to 740 postacetabular; posterior testis 182 to 259 by 155 to 217, 790 to 1,195 postacetabular; cirrus sac 179 to 345 (longitudinal extent) by 95 to 177, equatorial with anteriormost margin 260 to 500 postacetabular, thick walled, muscular, containing seminal vesicle, short pars prostatica, prostate cells and long, spined cirrus; seminal vesicle saccular when distended with sperm to somewhat tubular and winding, occasionally appearing bipartite; genital pore 375 to 570 postacetabular; ovary 92 to 131 by 114 to 162; metraterm very thick walled, muscular, somewhat winding, commencing at anterior margin of anterior testis; five operculate eggs 104 to 111 by 63 to 77, few.

DISCUSSION: Tubangui and Masiluñgan (1936) described this species from Luzon Island, Philippines, listing the host as a cobra, *Naja* sp. (Elapidae); later, Tubangui (1947) specifically identified the host as *Naja naja philippinensis* Taylor. Our report represents new host and geographic distribution records. Yamaguti (1958) and Skrjabin (1962) placed the genus *Harmotrema* Nicoll, 1914, in the family Liolopidae Dollfus, 1934, and subfamily Harmotrematinae Yamaguti, 1933; Mehra (1962) erected the family Harmotrematidae for this genus and *Helicotrema* Odhner, 1912. Our specimens show several variations from the original description of *H. eugari*. In our forms the left cecum is decidedly shorter than the right rather than nearly equal in length as illustrated, the acetabulum is at the level of the anterior one-fourth to one-third of body length rather than near the anterior one-sixth, the intertesticular space is shorter than the length of either testis rather than longer, the testes may be wider than long rather than always longer than wide, the cirrus sac is equatorial rather than preequatorial, the seminal vesicle may be saccular to tubular and coiled, occasionally bipartite in appearance, rather than saccular as illustrated, and the metraterm commences at the anterior margin of the anterior testis and is winding rather than anterior to this level and being straight as illustrated.

### FAMILY OPHIODIPLOSTOMATIDAE

### *Proalarioides kobayashii* Park, 1940

HOST: *Dinodon rufozonatum* (Cantor) (Colubridae).

HABITAT: Small intestine.

LOCALITY: 12 miles southwest of Seoul, Korea.

DATE: 17 October 1961.

SPECIMENS: USNM Helm. Coll. No. 61707 (four slides with one specimen each).

MEASUREMENTS AND SOME PERTINENT DATA (based on 11 mature and three young adults, and 22 immature worms from one host, seven mature measured): Body 2,001 to 4,027 in total length; spherical forebody 1,089 to 2,078 by 1,035 to 2,048; cylindrical hindbody 897 to 2,038 by 495 to 1,380; anterior extremity of body to acetabulum 391 to 620; posttesticular space 468 to 1,598; postcecal space 376 to 1,414; oral sucker 90 to 148 by 140 to 210; acetabulum 115 to 175 by 148 to 197; tribocytic organ 475 to 767 by 422 to 843; pharynx 106 to 136 by 70 to 116; esophagus 36 to 175 long; testes 34 to 178 apart; anterior testis 152 to 250 by 213 to 302, 552 to 2,294 postacetabular; posterior testis 150 to 230 by 206 to 360, 736 to 2,669 postacetabular; hermaphroditic sac present; ovary 97 to 194 by 111 to 195, 499 to 2,117 postacetabular; 17 operculate eggs 98 to 114 by 48 to 65.

DISCUSSION: Park (1940) described this species from the vicinity of Seoul, Korea, from *Elaphe dione* (Pallas) (Colubridae), *Natrix tigrina* (Boie) (Colubridae), and *Ancistrodon blomhoffi brevicaudus* Stejneger (Crotalidae). Our report is a new host record. Yamaguti (1958) placed the genus *Proalarioides* Yamaguti, 1933, in the family Proterodiplostomidae Dubois, 1936, and subfamily Ophiodiplostominae Dubois, 1936; Sudarikov (1960) erected the family Ophiodiplostomatidae and subfamily Proalarioidinae for this genus. Six of our seven measured specimens are shorter in total length than the shortest noted by Park. In spite of this the postcecal space in our forms

is much longer, averaging 826 microns, rather than being between 240 to 250. Additionally, the acetabulum is located much farther posteriorly, averaging 513 microns from the anterior extremity of the body, rather than being between 140 to 150.

FAMILY DICROCOELIIDAE

*Paradistomum gregarium* Tubangui, 1929

SYNONYMS: *Paradistomum magnum* Tubangui, 1928, nec Travassos, 1919; *Paradistomoides gregarium* (Tubangui, 1929) Travassos, 1944.

HOSTS: *Hemidactylus frenatus* Dum. and Bibr., *Platyurus platyurus* (Schneider) (Gekkonidae).

HABITATS: Gall bladder, liver.

LOCALITIES: Tacloban, Leyte Island (*H. frenatus*, *P. platyurus*); Clark Air Force Base, Luzon Island (*P. platyurus*); Philippines.

DATES: 14 (Leyte), 21 (Luzon) December 1961.

SPECIMENS: USNM Helm. Coll. No. 61708 (four slides with one specimen each from *H. frenatus*); No. 61709 (five slides with one specimen each from *P. platyurus*).

DISCUSSION: Our collection consisted of four, five, six, and seven mature adult worms from four *H. frenatus*, and one (mature adult, in two), three (mature adult), four (mature adult), 14 (two mature and nine young adult, three immature), and 17 (seven mature and 10 young adult) from six *P. platyurus*. The measurements and data, including extensive morphological variations, for the present specimens are essentially similar to those reported by us (1964, 1965) from *H. frenatus* from Palawan Island (Philippines) and North Borneo (Malaysia). This species was originally described from *H. frenatus* from Luzon, and redescribed from *H. gleodovi* Murray from Burma; we (1965) found it in *Gehyra mutilata* (Wiegmann) (Gekkonidae) from North Borneo. Its presence in *Platyurus platyurus* and location on Leyte represent new host and geographic distribution records.

FAMILY LECITHODENDRIIDAE

*Postorchigenes ovatus* Tubangui, 1928

SYNONYMS: *Palitrema macrorchis* Gogate, 1939; *Postorchigenes macrorchis* (Gogate, 1939).

HOST: *Hemidactylus frenatus* (Gekkonidae).

HABITAT: Stomach.

LOCALITY: Clark Air Force Base, Luzon Island, Philippines.

DATE: 15 December 1961.

SPECIMENS: USNM Helm. Coll. No. 61710 (three slides with one specimen each).

Measurements and some pertinent data (based on three specimens from one host): Body 1,565 to 1,882 by 815 to 950; spines sparser posteriorly, lacking at extreme posterior end of body; forebody 415 to 515, hindbody 958 to 1,195, previtellarian space 202 to 295, posttesticular space 480 to 705, postcecal space 225 to 410; longitudinal rows of subcuticular parenchymal gland cells in forebody; oral sucker 157 to 169 by 157 to 172; acetabulum 157 to 170 by 152 to 160, center at level of anterior three-tenths to one-third of body length; sucker length ratio 1:0.99 to 1.02; prepharynx present, short; pharynx 76 to 94 by 61 to 75; esophagus sinuous, as long as or longer than pharynx; ceca conspicuous because of very thick cell lining, ventral to testes; left (anterior) testis 390 to 440 by 295 to 385, right (posterior) testis 390 to 450 by 378 to 398; cirrus sac 254 to 290 by 140 to 145, relatively thin walled; pars prostatica with conspicuous cell lining; genital atrium shallow; genital pore in one worm slightly posterior to level of posterior margin of acetabulum, overlying anterior part of left testis and lying anterior to posterior margin of acetabulum in other two; ovary 170 to 220 by 205 to 245; uterus may extend anteriorly as far as anterior margins of ovary and acetabulum; metraterm long, sinuous, thick walled, muscular, surrounded by prominent gland cells; 15 operculate eggs 19 to 23 by 11 to 13.

DISCUSSION: Tubangui (1928) described this form as a new genus and species from the same host and island as our specimens. The latter show several variations from the original description. In our forms the acetabulum is about the same size as the oral sucker rather than being smaller, the esophagus is relatively long rather than being "very short, almost nil," the genital pore may be posterior to the level of the posterior margin of the acetabulum rather than anterior to this margin, and the testes are larger in size while the ovary is the same size or smaller. Tubangui

made no mention of the presence of a prepharynx or metraterm. There are two other recognized species in the genus: *P. macrorchis* (Gogate, 1939) (syn. *Palitrema m.* G.) from lizards, *Hemidactylus brooki* Gray (Gekkonidae) and *Calotes versicolor* (Daudin) (Agamidae) from Burma, and *H. flaviviridis* Rueppell from India; *P. duboisi* Rohde, 1963, from a bat, *Cheiromelas torquatus* Horsfield (Molossidae), from Malaya. Our material fits the descriptions of *P. macrorchis* given by Gogate (1939) and Baugh (1957). The specimens illustrated by the former from *H. brooki* and the latter from *H. flaviviridis* are more like ours than is that shown by Tubangui. The longitudinal rows of gland cells noted by Gogate and by Baugh were seen only in part in one of our specimens. It has been our experience and that of other investigators that the detection of parenchymal glands when present depends upon the method employed for preparation of the whole mounts and are not always demonstrated. We declare *Postorchigenes macrorchis* (Gogate, 1939) a synonym of *P. ovatus*.

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## *Presentations*

# 1966 ANNIVERSARY AWARDS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

### 421st Meeting 19 October 1966

George Roger LaRue, whom we honor at this meeting with an Anniversary Award of the Helminthological Society of Washington, is recognized as one of the most influential and respected American zoologists and parasitologists of his generation.

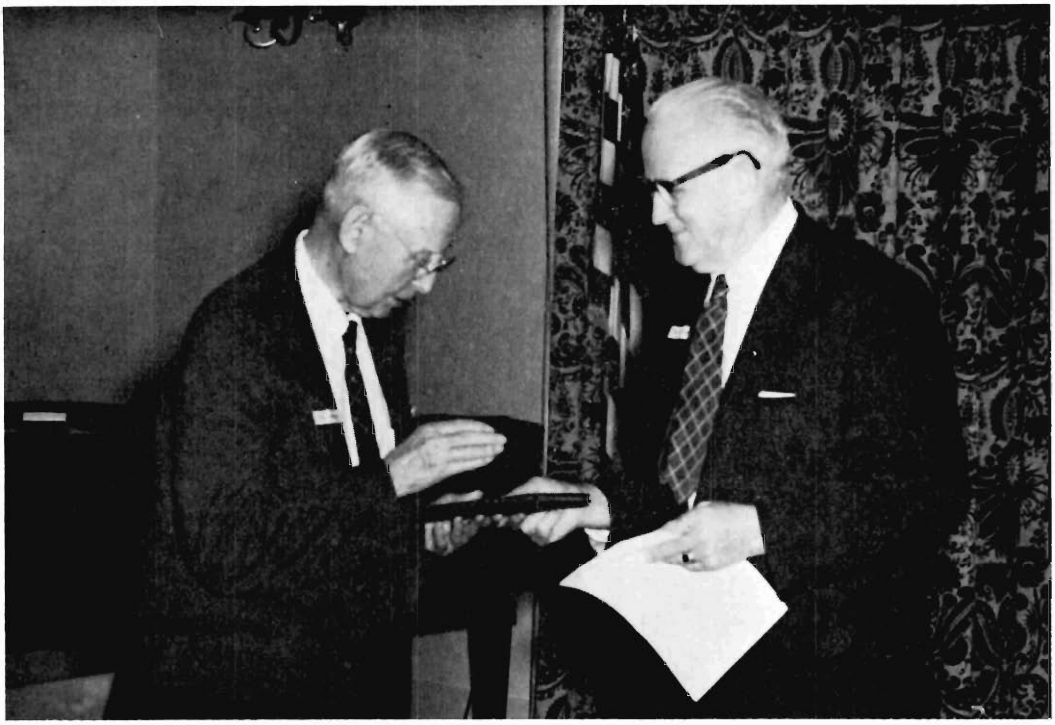
Born in Paullina, Iowa (1882), he was graduated from Doane College (B.S., 1907) and received his graduate training at the University of Nebraska (A.M., 1909) and the University of Illinois (Ph.D., 1911) under Professor H. B. Ward.

After service as Assistant Zoologist, Doane College (1905–1907), Technician, Medical College, University of Nebraska (1907–1909), and Research Assistant and Assistant Zoologist, University of Illinois (1909–1911), he embarked on a distinguished career at the University of Michigan. It began in 1911 and officially spanned 41 years. He was Professor of Zoology for 25 years, Chairman of the Zoology Department for 15 years and Director of the University's Biological Station at Douglas Lake for 23 years. He was appointed Visiting Investigator, Zoological Division, U. S. Department of Agriculture, Beltsville, Maryland, in 1951, Emeritus Professor of Zoology, University of Michigan, in 1952, and Visiting Professor of Parasitology, Rice Institute, for 1952–1953. Among other prior positions were Science Assistant and Assistant Zoologist, U. S. Bureau of Fisheries, honorary Curator and Research Associate, University of Michigan Museum, and Fellow by Courtesy, School of Hygiene and Public Health, Johns Hopkins University (1924–1925).

He received an American Medical Association honorary Research Grant and an honorary Sc.D. from Doane College (1947).

During his long tenure at Michigan, he trained and served as advisor to numerous graduate students who subsequently achieved leadership as educators and researchers in zoology and parasitology. While affiliated with the U. S. Department of Agriculture at Beltsville, he was a valued consultant and advisor to his associates, ably reviewed many of their research manuscripts and presented an eminently mature graduate course in trematodology in the Departments' Graduate School.

Much of his research, which resulted in many valuable contributions to knowledge of the morphology, taxonomy, and life histories of tapeworms and digenetic trematodes, was conducted at the Michigan Biological Station where his prominent position caused students and faculty to name him "Governor of Northern Michigan."



Dr. George Roger LaRue (left) receiving a 1966 Anniversary Award of the Helminthological Society of Washington (presented by A. McIntosh).

Previously honored with the presidency of the American Microscopical Society and the vice-presidency and presidency of the American Society of Parasitologists, he ably served as Editor of the *Journal of Parasitology* in 1954 and 1955, when more than three-score and ten, and is still a Consultant to the Editor.

Early in 1913, together with such notables as Theobald Smith and S. T. Darling, he was elected an American corresponding member of the Helminthological Society, then just over two years old. In 1924 he participated in the actions of the Society which resulted in the founding of the American Society of Parasitologists. He has regularly participated in affairs of the "Helm.-Soc." since 1951 and was elected an Honorary member in 1959.

To honor him for his far-reaching influence as an educator and molder of the professional careers of now leading zoologists and parasitologists in this country and abroad and his accomplishments in helminthological research and in token of our esteem for him and appreciation of his 53 years of loyal membership, The Helminthological Society of Washington is privileged to present to George Roger LaRue this Anniversary Award. (Committee: Lucker, Lund, Sadun. Presentation: Allen McIntosh.)

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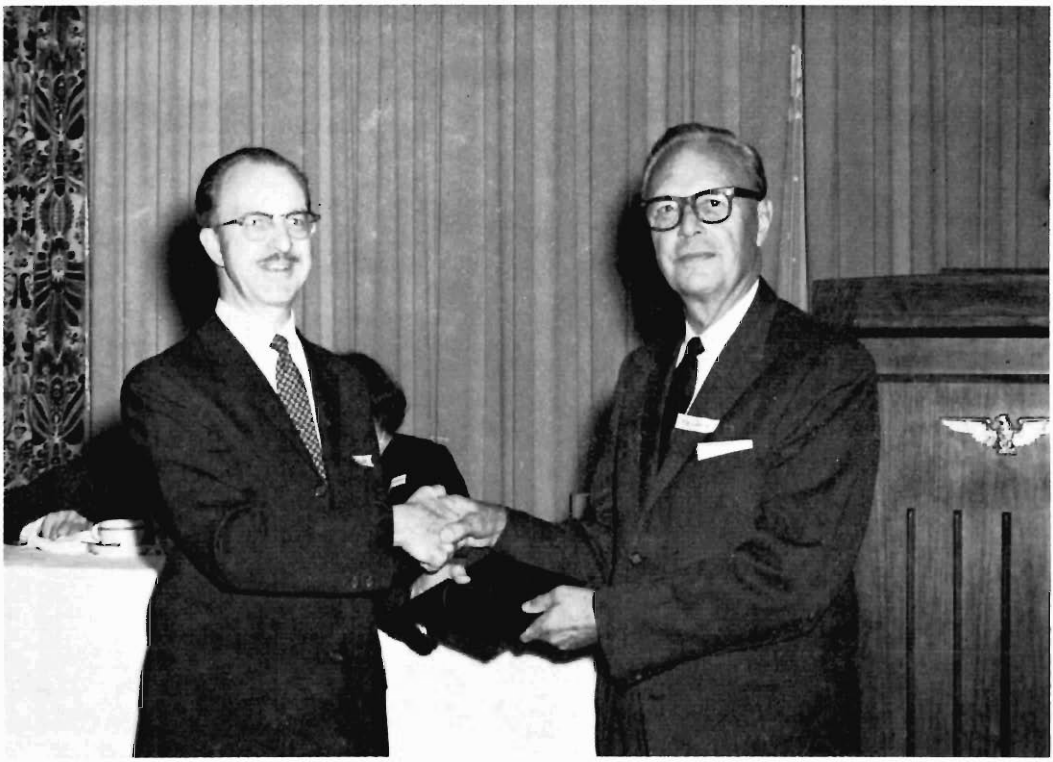
William Walter Cort, now to receive an Anniversary Award of the Helminthological Society of Washington, is noted and esteemed internationally as a medical helminthologist and parasitologist.

He was born in Leon, Iowa (1887), is a graduate of Colorado College (1909), and received an A.M. (1911) and a Ph.D. degree (1914) in Zoology from the University of Illinois, where he studied under Professor Henry B. Ward.

He was Instructor in Zoology at Colorado College (1912–1913), Professor of Zoology at Macalester College (1914–1916), Assistant Professor of Zoology at the University of Cali-



Dr. L. J. Olivier (left) accepting on behalf of Dr. William Walter Cort a 1966 Anniversary Award of the Helminthological Society of Washington (presented by J. T. Lucker).

fornia (1916–1919), and in the School of Hygiene and Public Health, Johns Hopkins University, became Associate Professor of Helminthology (1919–1925), Professor of Helminthology (1925–1943), Professor of Parasitology (1943–1953) and Emeritus Professor (1953–). He was appointed Research Professor (1953–1959) and Emeritus Research Professor (1959–) in the School of Public Health, University of North Carolina.

An honorary Sc.D. was awarded to him by the University of North Carolina (1946) and by Colorado College (1949).

Among other positions in which he also served were: Consultant Helminthologist, California State Board of Health; Visiting Professor, Peking Union Medical College, China; Lecturer, School of Public Health, Harvard University; Tropical Medicine Consultant, U. S. Secretary of War; Consultant, Laboratory Division, Communicable Disease Center, U. S. Public Health Service; Director, Program of Study of Ascariasis in Children, National Research Council and American Child Health Association; Member, Commission on Hookworm Diseases, International Health Division, Rockefeller Foundation.

During his tenure at Johns Hopkins, dozens of graduate students earned a doctorate under his tutelage and guidance. Many subsequently achieved leadership in research in medical or veterinary helminthology.

Also notable is his enviable record of research accomplishments in helminthology. He has investigated the morphology, embryology and life cycles of trematodes, hookworm disease, ascariasis, schistosomiasis, and many other problems. He is the author, or a coauthor of about 200 parasitological books, monographs, papers and notes published from 1912 to 1960.

A member of several professional societies and active in all, he was honored with the

presidency of four, among them the American Society of Parasitologists, which he also served as Editor and in almost all other official capacities.

Elected a member of "Helm.-Soc." in 1920, he has since been an active participant in its affairs. He was elected President for 1924–1925 and a life member in 1954. Apparently, he sometimes made the roundtrip from Baltimore to Washington meetings by railroad and streetcar! He and his associates at Johns Hopkins were hosts for all November meetings from 1920 through 1934 and for many subsequent Baltimore meetings; often supper was provided for all comers. He induced many of his students to join the Society and was a member of the "Helm.-Soc." committee which implemented the founding of the American Society of Parasitologists and even provided the constitution adopted by the founders in 1924.

In recognition of his high rank as an authority, educator, researcher, and instigator of research, in medical helminthology and in grateful appreciation of his contribution to this Society's stature, welfare and growth, The Helminthological Society of Washington proudly presents to William Walter Cort this Anniversary Award. (Committee: Lucker, Lund, Sadun. Presentation: J. T. Lucker).

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*In Memoriam*

**MOHAMMAD ABDUL BASIR**

**Aligarh Muslim University**

**1916–1966**

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