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PROCEEDINGS OF THE
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VOLUME 38 1971 NUMBER 2

The Secretory Nature of the Excretory Gland Cells of *Stephanurus dentatus*. I. Morphology and Histochemistry

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ABSTRACT: Morphological, histochemical, and ultrastructural studies show that the excretory gland cells of *Stephanurus dentatus* contain granules resembling the secretory granules of various exocrine and endocrine glands. The granules are eosin and PAS positive. Glycogen, acid mucopolysaccharides, and lipids were not detected histochemically. Therefore, the granules are thought to contain glycoprotein. From this evidence it is postulated that the excretory gland cells have a secretory function.

Taylor (1900), Chitwood and Chitwood (1950), Enigk and Grittner (1952), Tromba and Baisden (1964), Douvres, Tromba, and Doran (1966), and Waddell (1968) have described the morphology of the excretory glands of adult and larval stages of the swine kidney worm, *Stephanurus dentatus* Diesing, 1839. However, these studies gave no evidence concerning the function of this gland. Weinstein's statement (1960) that "the so-called excretory system remains an enigma, and an interpretation of its function still depends on speculation," remains true. Therefore, morphological, histochemical, and ultrastructural studies were conducted to define the chemical nature and function of the excretory gland cells.

Material and Methods

Adult worms were collected from ureteral cysts of infected swine kidneys obtained from a packing house. Intact excretory glands were removed from the worms as described by Tromba and Baisden (1964). Depending upon the procedure to be employed for staining, whole worms and intact excretory glands were either fixed in Helly's solution or frozen.

For light microscopy, paraffin embedded serial sections were cut at 6 μ and subjected to the following procedures according to Humason (1962): (1) hematoxylin and eosin stain; (2) Gomori's trichrome stain and Himes and Moriber triple stain for proteins, carbohydrates, and nucleic acids; (3) PAS both with and without 1% malt diastase digestion for carbohydrates; (4) the Bauer-Feulgen reaction for glycogen; and (5) the Feulgen reaction for DNA. Frozen sections were cut on an IEC Cryostat at 10 μ and subjected to the following procedures according to Barka and Anderson (1965): (1) alcian blue, toluidine blue, and methylene blue for acid mucopolysaccharides; (2) sudan black B and oil red 0 for lipids; (3) naphthol AS-TR phosphate with fast red violet LB as coupler for alkaline phosphatase; and (4) naphthol AS-BI phosphate with pararosaniline-HCl as coupler for acid phosphatase.

For electron microscopy both whole worms and intact excretory glands were immersed in cold 3% glutaraldehyde (cacodylate buffer 0.1 M; pH 8.0) for 12 hr, post-fixed in cold 1% osmium tetroxide for 1 hr, and embedded in Epon. Tissue sections were stained with uranyl acetate and lead citrate, then examined in an AEI EM6B electron microscope.

Results

Morphology

The excretory system of *S. dentatus* is of the rhabditoid type and consists of pore, duct, sinus, paired lateral canals, and a pair of sub-

ventral gland cells each filled with spherical bodies. These spherical bodies, called granules by Tromba and Baisden (1964) and corpuscles by Enigk and Grittner (1952), are 2–4 μ in diameter. A large ovoid nucleus averaging 490 by 1,570 μ is found in the lower part of each gland cell well below the sinus area. The nucleus consists of a homogeneous material. No nucleolus can be seen by light microscopy or by the histochemical methods used. Sinus nuclei are readily observable (Figs. 1, 2).

Figures 1–4 show the relationship between the excretory sinus and the excretory gland cells as one proceeds posteriad in the gland cell. Anteriorly, the sinus opens to the terminal duct, and posteriorly extends into the subventral glands and terminates just distal to the esophago-intestinal valve. A membrane separates the granules of the gland cell from the excretory sinus (Fig. 4). The sinus is connected to the lateral canals by branches at the level where the intestine overlaps the esophagus (Figs. 5–7). The lateral excretory canal divides into two branches which are embedded in the lateral chords. The anterior branch extends to the base of the buccal capsule, and the posterior branch extends nearly to the tail end of the worm. Figure 8 shows an overall view of the connection between the lateral excretory canals and the excretory sinus of the gland cells.

Histochemistry

The excretory pore is lined with a collagen-like protein, characteristic of cuticular tissue, as evidenced by the positive reaction with the Himes and Moriber, and Gomori's trichrome stains. The excretory duct, excretory sinus, lateral excretory canals, and cytoplasm of the gland cells gave positive reactions for protein with the above stains. The granules are eosin-positive, PAS-positive both with and without 1% malt diastase digestion, and exhibit a pink to red color with the Himes and Moriber, and Gomori's trichrome stains. The excretory gland cell cytoplasm was PAS-positive, 1% malt diastase digestion removed this PAS-positive reaction, thus indicating the presence of glycogen. Glycogen was also detected in the excretory gland call cytoplasm by the Bauer-Feulgen reaction but was not detected in the granules. Each gland cell nucleus and the nuclei in the excretory sinus gave a positive Feulgen reaction for DNA. None of the structures stained for lipids using sudan black B and oil red 0, nor for acid mucopolysaccharides using alcian blue, toluidine blue, and methylene blue below pH 4.0, or reacted for acid or alkaline phosphatase.

Although the excretory gland cells did not stain for lipids, thin layer chromatography on silica gel G of a chloroform–methanol (2-1 v/v) extract of the gland cells showed the presence of mono, di, and triglycerides, free fatty acids, phospholipids, and sterols.

Electron and microscopy

Electron microscopy revealed the presence of free ribosomes, rough endoplasmic reticulum, mitochondria, glycogen particles, and secretory granules (Figs. 9, 10). The granules have a limiting membrane and are electron dense.

→

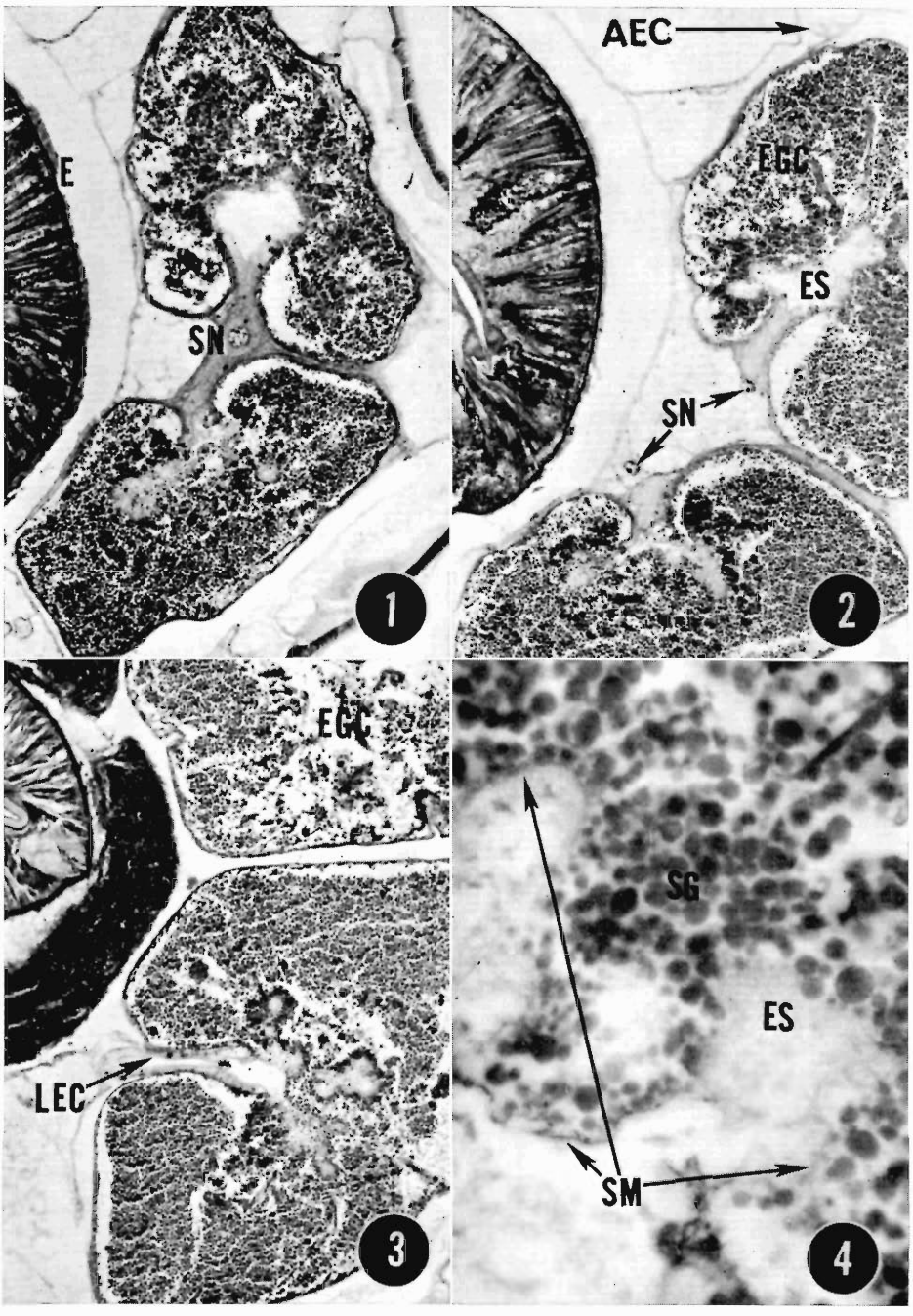
Abbreviations: Anterior branch of excretory canal (AEC), esophagus (E), excretory gland cell (EGC), excretory sinus (ES), lateral excretory canal (LEC), secretory granules (SG), sinus membrane (SM), sinus nucleus (SN).

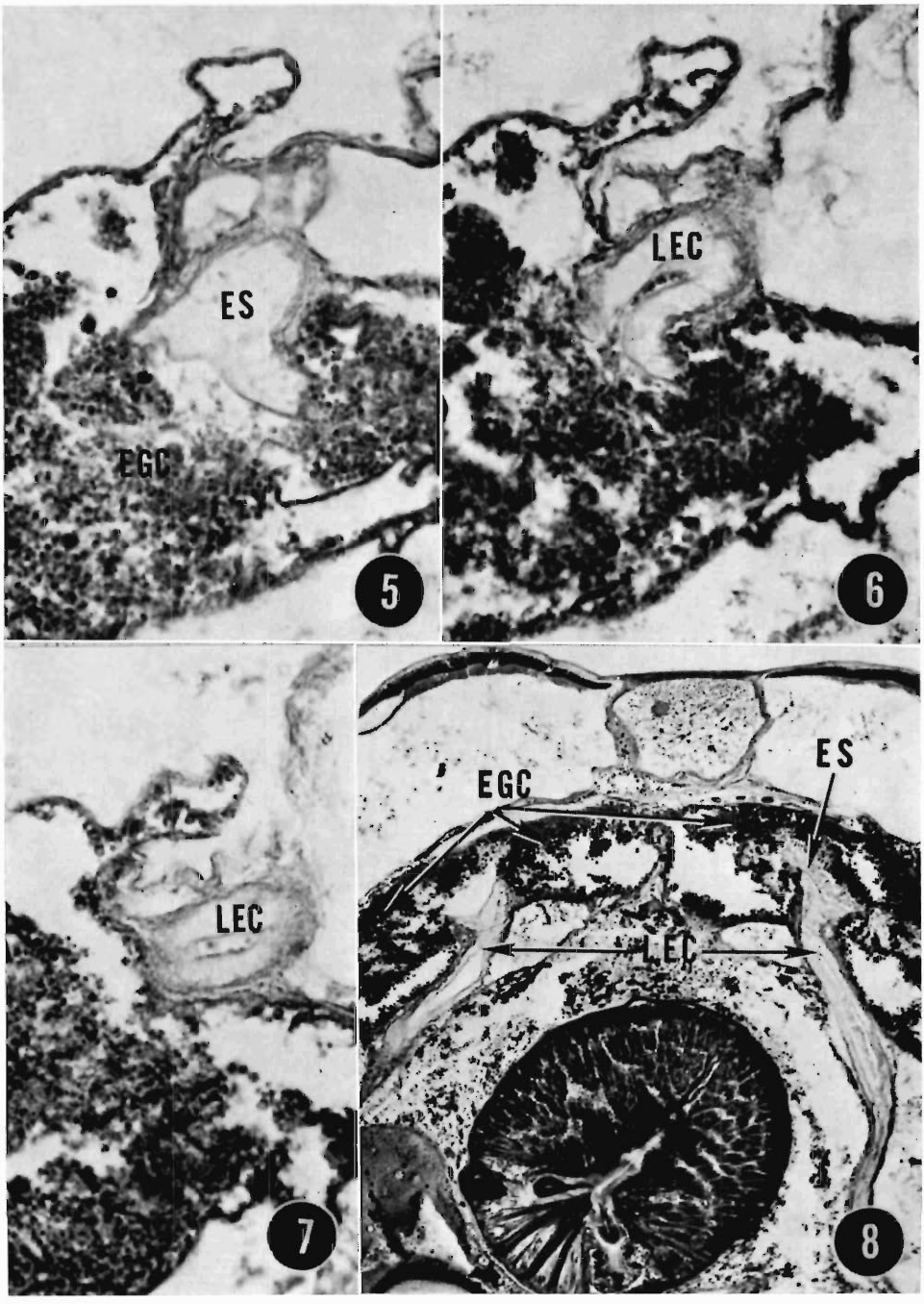
Figures 1–4. Transverse serial sections of an adult *Stephanurus dentatus* showing the relationship between the excretory sinus and the excretory gland cells. 1. Both gland cells connected in the area of the sinus bridge. × 165. 2. A more posterior view showing that the gland cells have begun to separate. × 165. 3. The gland cells have completely separated. × 165. 4. Sinus area deep in the gland cell. × 1,250.

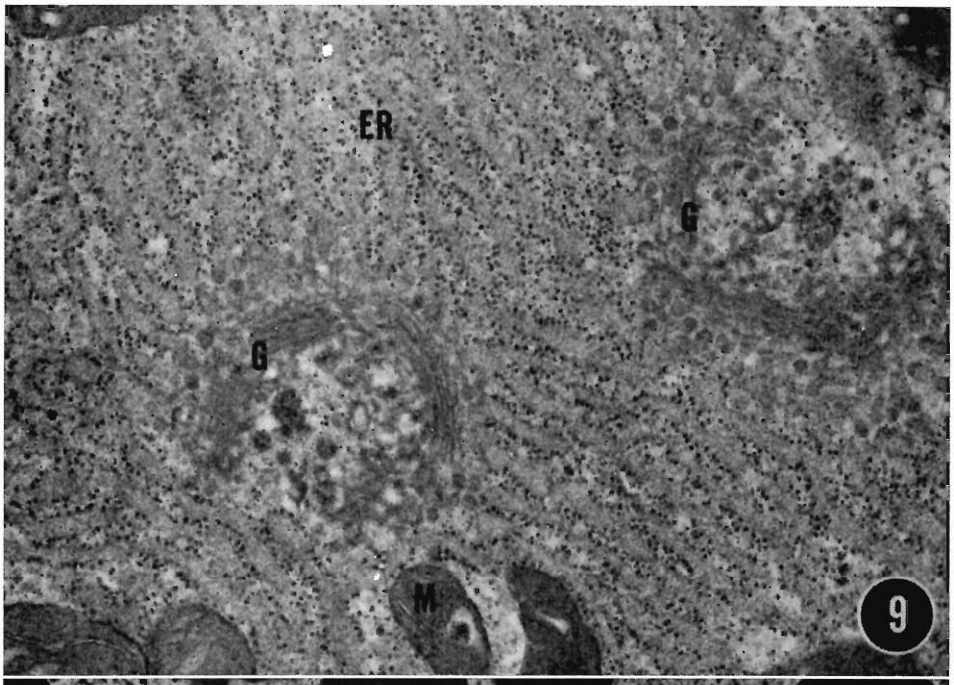
Figures 5–7. Transverse serial sections of an adult *Stephanurus dentatus* showing the formation of the lateral excretory canal from the excretory sinus. × 620. 5. Lateral excretory canal just *starting to form.* 6. Lateral excretory canal almost formed. 7. Lateral excretory canal completely formed.

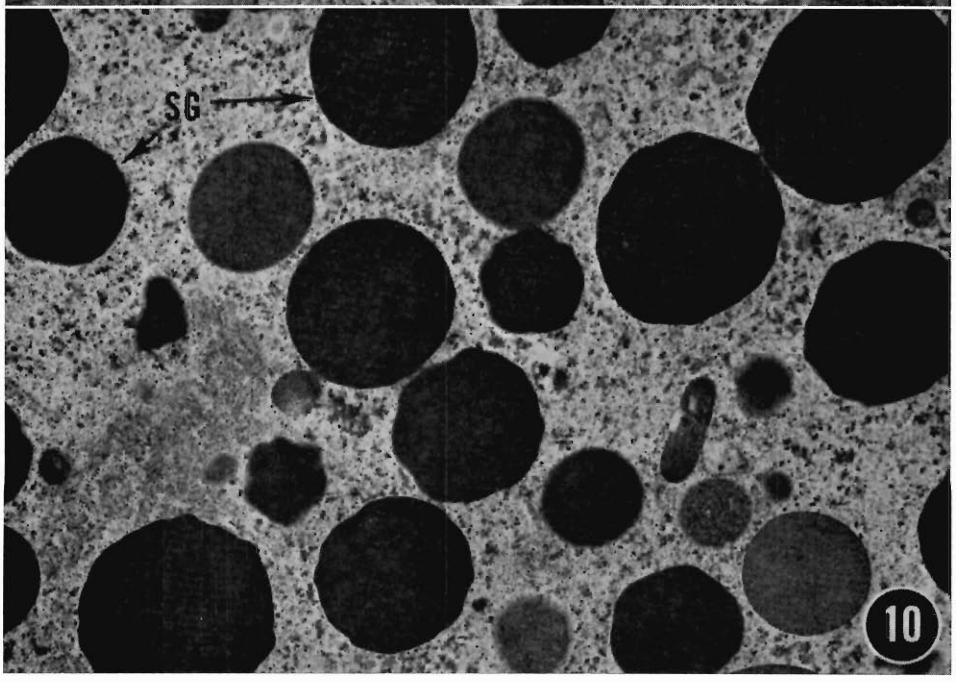
Figure 8. Transverse section of an adult *Stephanurus dentatus* showing both lateral excretory canals, excretory sinus, and gland cells. × 165.

Figures 9, 10. Electron micrographs of the excretory gland cell of *Stephanurus dentatus*. 9. Section showing mitochondria (M), rough endoplasmic reticulum (ER), and Golgi bodies (G). × 30,000. 10. Section showing the secretory granules (SG). × 10,000.









Discussion

In general, the gross morphological findings agreed with those of previous investigators. However, Waddell (1968) reported that the lateral canals extend only half the length of the body, whereas we found that they extend nearly to the posterior end of the worm.

No reports on the excretory system of *S. dentatus* mention the membrane which separates the sinus area from the granules of the gland cell. The granules do not pass beyond this membrane, as no granules are seen in the terminal duct, excretory sinus, or lateral canals. A material staining red with the Himes and Moriber triple stain is observable in the terminal duct and excretory pore, and appears similar to that found in the granules. We postulate that the granules rupture or lyse at the membrane surface, and release their contents into the sinus area. From here, the material can move up into the terminal duct and eventually out of the excretory pore, or it can move into the lateral canals for distribution to other parts of the worm.

The excretory gland cells are composed mainly of protein as evidenced by the histochemical stains. Since lipids are not detected histochemically but are detected by thin layer chromatography, they are probably in a bound state and thus not free to react with the lipid stains. Glycogen is present in the cytoplasm of the gland cells but not in the granules. Because the granules are eosin and PAS positive, toluidine blue and alcian blue negative, and do not bind methylene blue below pH 4.0, we regard the material inside the granule as a glycoprotein.

Electron microscopy of the gland cells of *S. dentatus* revealed cellular components characteristic of various exocrine and endocrine glands. Morphologically, histochemically, and ultrastructurally the granules resemble the secretory granules of the guinea pig pancreas (Siekevitz and Palade, 1957), Brunner's glands of the mouse (Friend, 1965), the excretory vesicle of *Cryptocotyle lingua cercaria* (Krupa, Cousineau, and Bal, 1969), the pharyngeal glands of *Enchytraeus albidus* (Reger, 1967), and the anterior pituitary of the bovine (Tesar, Koenig, and Hughes, 1969). Therefore, we conclude that the granules of the excretory gland cells of *S. dentatus* are secretory granules, and that the gland cells have a secretory function.

Acknowledgments

We are grateful to Mrs. M. B. Chitwood for her advice and generous assistance, and to Drs. L. A. Baisden, F. W. Douvres, and F. G. Tromba for their interest and counsel during this investigation. We wish to thank N. S. Dittemore, A. W. Jones, J. M. Lindemulder, G. M. Malakatis, and M. L. Rhoads for technical assistance. We also wish to thank Dr. J. M. Vetterling and H. R. Waldrop for assistance in the histochemical work. The authors thank Smithfield Packing Co., Smithfield, Va.; Briggs and Co., Landover, Md.; and the Meat Inspection Division, ARS, USDA, Washington, D.C., for supplying the swine kidneys.

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Observations on the Life Cycle of *Pharyngostomoides* spp. and the Description of *P. adenocephala* sp. n. (Strigeoidea: Diplostomatidae) from the Raccoon, *Procyon lotor* (L.)[1]

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ABSTRACT: Two species of *Pharyngostomoides* Harkema, 1942, were included in the original description of *Pharyngostomoides procyonis* Harkema, 1942. A redescription of *P. procyonis* is presented and *Pharyngostomoides adenocephala* sp. n. is described. Both species utilize the snail, *Menetus dilatatus buchanensis* (Lea), as the first intermediate host and the raccoon, *Procyon lotor* (L.), as the definitive host. The two species show morphological differences in the daughter sporocyst, cercaria, and adult.

Studies in this laboratory have shown that two adult forms of the genus *Pharyngostomoides* Harkema, 1942, can be separated on the basis of size, shape, presence or absence of glands around the pseudosuckers, and presence or absence of an ejaculatory pouch. Further pronounced differences were noted in the life histories of these two forms. Harkema and Miller (1964) observed these two distinct forms and remarked that further studies might reveal two species. Harris, Harkema, and Miller (1967) reported the maternal transmission of *P. procyonis* Harkema, 1942, but stated that the genus contained more than one species.

Materials and Methods

The snail host for both species is *Menetus dilatatus buchanensis* (Lea). Specimens were collected locally and laboratory reared according to the methods of Fried and Goodchild (1963).

Raccoons were live-trapped; the two species of worms were removed from the small intestine and separated. Adult worms were prepared as whole mounts, sectioned, or teased for ova. Ova were concentrated, cleaned, and allowed to develop in pond water in finger bowls at room temperatures. After initial hatching the remaining ova were refrigerated until needed. Hatching was stimulated by strong light at room temperatures. Live miracidia were studied unstained and stained with Nile blue sulfate or neutral red and mounted in egg white for morphological studies. Miracidia used for measurements were killed and fixed in 5% formalin. The epidermal plate count was determined after the method of Lynch (1933). Experimental infection of snails was effected by exposing each uninfected snail

[1] Contribution from the Zoology Department, North Carolina Agricultural Experiment Station, Raleigh, N.C. Published with the approval of the Director of Research as Paper No. 3292 of the Journal Series.

to 2 or 3 miracidia in a 27 mm watch glass containing pond water.

Laboratory-reared snails have transparent shells which permitted observation of developing sporocysts. Sporocysts were recovered from crushed snails and studied in the same manner as the miracidia except that measurements were made on stained and mounted specimens.

Water from cultures of exposed snails was examined daily to determine cercarial emergence. Infected snails were isolated and examined at various times of the day to determine numbers and emergence habits of cercariae. Cercariae were studied alive, stained, and unstained in the same manner as the miracidia.

All measurements are in microns unless otherwise stated.

Results and Discussion

Among the intestinal parasites of the raccoon, two types of flukes in the genus *Pharyngostomoides* can be distinguished. There is a large, broad, pinkish form (to be described below) and a smaller, narrower, ivory-colored form. The measurements by Harkema (1942) for *P. procyonis* include both forms but those by Chandler and Rausch (1946) for *P. ovalis* are for the smaller form. The description of *P. ovalis* is similar to that given by Harkema (1942) for "young" *P. procyonis*. The type specimen (No. 44850) of *P. procyonis* on deposit in the U. S. National Museum is the "smaller" form, hence *P. ovalis* is reduced to a synonym. The generic description of *Pharyngostomoides* is still valid except for the reference to young specimens. This description differs from that by Dubois (1966) in that it does not include members of the genus *Parallelorchis* Harkema and Miller, 1961. Dubois' synonymy of *Parallelorchis* and *Pharyngostomoides* is not accepted for the reasons given by Harkema and Miller (1961).

Pharyngostomoides procyonis, adult (Figs. 1, 4)

Description (based on 12 specimens): Body small, 0.76–1.10 mm in length. Forebody 440–500 long by 400–450 wide, scoop-shaped, with lateral margins folded ventrally. Forebody longer than hindbody. Anterior two-thirds of forebody covered by small spines. Hindbody conical 315–560 by 245–425. Oral sucker subterminal, slightly broader than long, 55–75 by 58–90. Pseudosuckers present. Glands, if present, weakly developed. Acetabulum 65–75 by 65–100, lying near intestinal bifurcation, often obscured by holdfast organ. Holdfast well developed, 175–380 by 185–340 with longitudinal slitlike opening. Prepharynx short. Pharynx 50–85 by 40–60. Esophagus short, bifurcation of gut just anterior to level of acetabulum, ceca extend to near posterior end of body.

Testes two, spherical to ovoid, usually contiguous, symmetrical, but either may be slightly displaced posteriorly, 165–255 by 100–225. Seminal reservoir is enlarged, convoluted, ventral, and posterior to ovary. Vas deferens expanded posterior to testes as seminal vesicle. Latter empties into thick-walled muscular ejaculatory pouch which unites with uterus forming hermaphroditic duct in genital cone. Latter may or may not extend beyond dorsal surface of hindbody. Genital pore on genital cone. Posterodorsal genital atrium well developed.

Ovary reniform, elongated transversely, located equatorially in body, 65–100 by 105–160. Oviduct arises from posterodorsal surface of ovary, gives off Laurer's canal and passes into Mehlis' gland. Uterus has short ascending loop, then passes posteriorly to unite with ejaculatory duct as short hermaphroditic duct. Vitellaria follicular, in forebody and in hindbody from acetabulum posteriorly to anterior margins of testes, extending slightly into holdfast organ. Vitelline reservoir in midline; common vitelline duct empties into oviduct just proximal to junction of oviduct and Laurer's canal. Ova not numerous, 80–104 by 55–67. Excretory pore subterminal on posteroventral surface of hindbody.

Host: *Procyon lotor* (Linnaeus).

Habitat: Small intestine.

Locality: Wake County, North Carolina; Angelina County, Texas.

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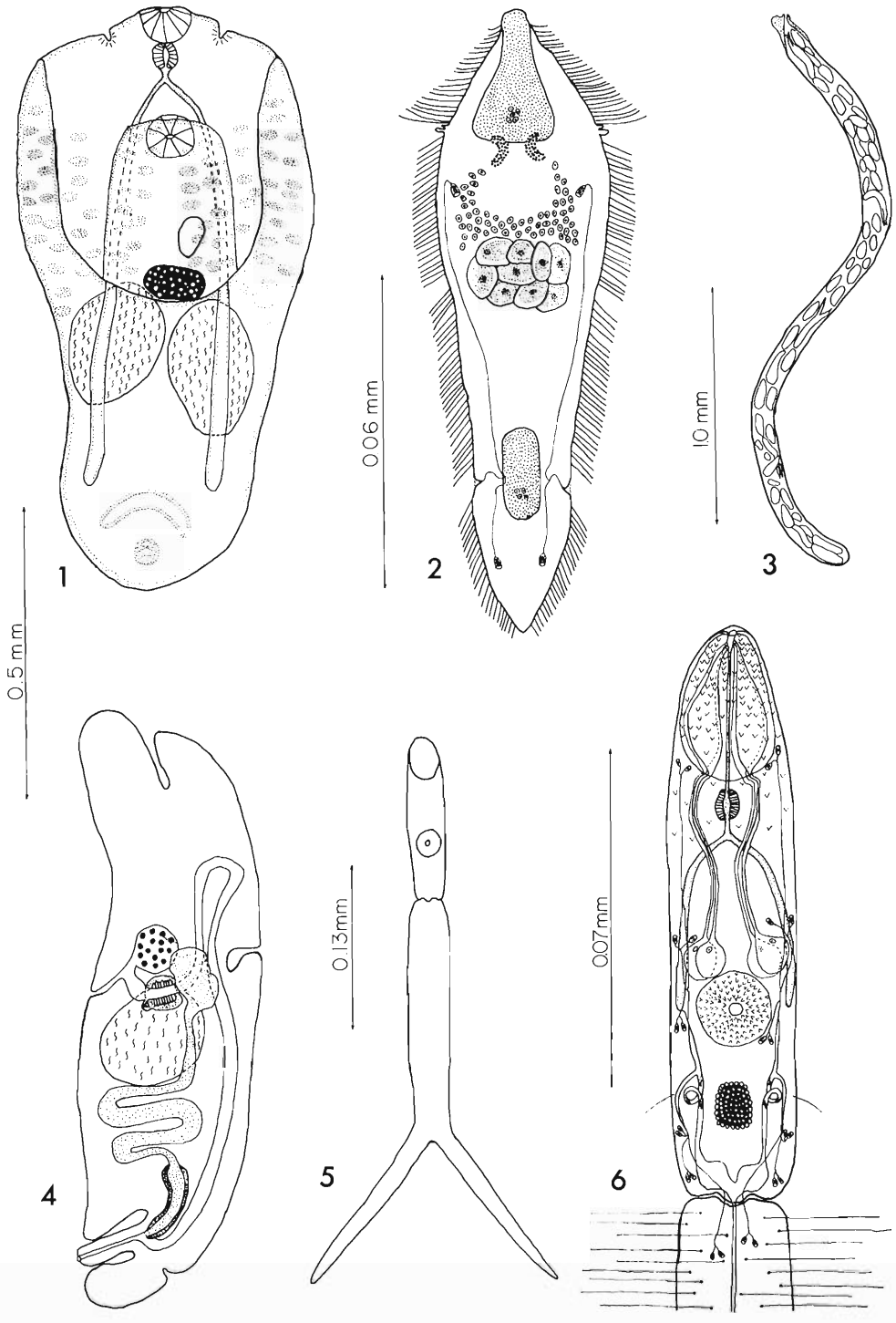
Figures 1–6. ***Pharyngostomoides procyonis*** **Harkema, 1942. 1. Adult, ventral view. 2. Miracidium, showing internal anatomy. 3. Daughter sporocyst, 18 days postinfection. 4. Adult, sagittal view showing prominent ejaculatory pouch. 5. Cercaria, showing shape. 6. Cercaria, detailed view of body features.**



SPECIMENS: USNM Helm. Coll. Nos. 44850 (Type); 44851 (Paratypes).

Pharyngostomoides adenocephala sp. n. (Figs. 7, 10)

DESCRIPTION (based on 12 specimens): Body small, 1.2–1.9 mm in length. Forebody 0.70–0.95 mm long by 1.05–1.26 mm wide, scoop-shaped, usually flexed dorsad. Two-thirds of forebody covered by small spines. Hindbody 525–850 by 600–900, conical. Oral sucker subterminal, 100–135 by 130–160. Pseudosuckers well developed, sometimes protruding as lappets, with masses of unicellular glands (Fig. 7). Acetabulum 120–150 by 120–160, located in anterior fourth of body just posterior to gut bifurcation. Holdfast well developed, 275–430 by 300–640, sometimes obscuring acetabulum.

Prepharynx very short, usually not discernible in whole mounts. Pharynx 100–110 by 80–95. Esophagus short; bifurcation of gut just anterior to acetabulum. Ceca extend to near posterior end of body.

Testes two, generally symmetrical, sometimes slightly asymmetrical, spherical to ovoid, usually contiguous, in anterior part of hindbody, 255–480 by 245–360. Seminal reservoir convoluted, near level of ovary. Vas deferens passes posteriorly between testes, expanding as convoluted seminal vesicle posterior to testes. No ejaculatory pouch, ejaculatory duct joining uterus to form hermaphroditic duct. Latter enters genital cone which may extend beyond dorsal body surface.

Ovary small, reniform, transversely elongated, in posterior part of forebody, 95–145 by 150–250. Oviduct arises dorsally, joined by Laurer's canal, next by vitelline duct, and then passes into Mehlis' gland. Uterus ascends anteriorly to level of holdfast then passes posteriorly between testes to hermaphroditic duct. Vitellaria follicular, in forebody from level of acetabulum to anterior margins of testes. Vitelline reservoir located at or near Mehlis' gland in middle of body. Common vitelline duct empties into oviduct just posterior to Laurer's canal. Ova few, 82–102 by 65–68 as measured alive and embryonated. Excretory pore subterminal on posteroventral surface of hindbody.

HOST: *Procyon lotor* (Linnaeus).

HABITAT: Small intestine.

LOCALITY: North Carolina.

SPECIMENS: USNM Helm. Coll. No. 71586 (holotype and paratypes), No. 71587 (paratypes —one whole mount and one frontal section).

Comparison of Adults

Living specimens of these two species can be separated by color, size, and shape. *P. adenocephala* is pinkish in color, larger, spatula-shaped, with the forebody flexed dorsad. *P. procyonis* is cream or ivory colored, smaller, more ovoid, with forebody and hindbody in the same plane. *P. adenocephala* has deep staining glandular masses associated with the pseudosuckers, hence the specific name; these are not evident in *P. procyonis*. *P. adenocephala* does not have a muscular ejaculatory pouch, whereas it is very evident in *P. procyonis*.

The differences in adult morphology of the two species are sufficient to warrant separation but differences in certain life history stages also substantiate the two species.

In a typical mixed infection the adults of *P. adenocephala* are not usually as abundant as *P. procyonis* and are more localized in the proximal portion of the duodenum. The latter species is present in the entire length of the duodenum.

Miracidia (Figs. 2, 8)

Observations on the miracidia of both species of *Pharyngostomoides* revealed no evident morphological differences. Hence the description given here is applicable to both.

Twenty-five miracidia fixed in hot formalin were 99–125 by 29–57. Apical papilla prominent. Lateral papillae bulbous. Epidermal plate arrangement 6,9,4,3. Small accessory plates (1 to 4) sometimes present. Eyespots pigmented, dorsal, at level between first and second tier of plates. Two pairs of flame cells,

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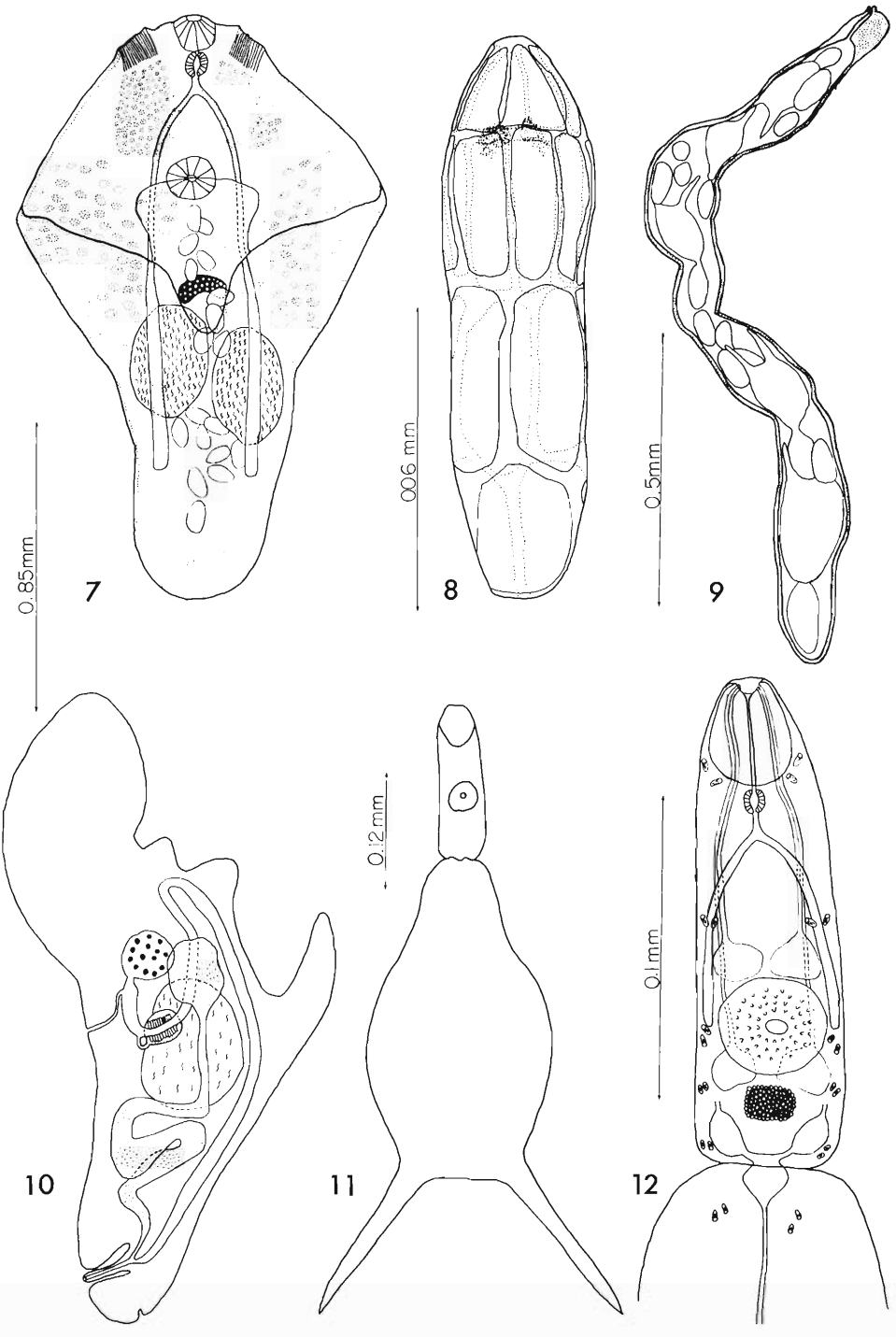
Figures 7–12. ***Pharyngostomoides adenocephala*** **sp. n. 7. Adult, ventral view. 8. Miracidium, showing pattern of epidermal plates. 9. Daughter sporocyst, 33 days postinfection. 10. Adult, sagittal view showing absence of prominent ejaculatory pouch. 11. Cercaria, showing shape. 12. Cercaria, detailed view of body features.**



lateral excretory pores between third and fourth tier of plates. Neural mass is a central non-staining spherical area enclosed by deeply staining cells. Eight to ten large germinal cells located posterior to neural mass. Multinucleated granular body (Pearson, 1956) present.

Ova begin to hatch after 11 days incubation at room temperature. Eyespots were visible after 7 days. Refrigerated eggs hatched six months after storage when stimulated by light at room temperatures. Miracidia of both species penetrate *Menetus dilatatus buchanensis*. Contact with the snail sppears to be random. The miracidium moves along the shell to the edge of the mantle where penetration is completed within 3–5 minutes.

Sporocysts (Figs. 3, 9)

Sporocysts of the two species of *Pharyngostomoides* cannot be distinguished except in mature daughter sporocysts containing developing cercariae. In both species the early mother sporocyst is in the mantle. As they develop they migrate to the periesophageal sinus where daughter sporocysts are liberated 7–12 days postinfection. Daughter sporocysts move to the digestive gland and can be seen through the thin, almost transparent shell of the living snail.

Early mother sporocyst

One mother sporocyst, 303 by 107, was recovered 5 days postinfection. It was ovoid in shape and the eyespots were well separated. The apical papilla was still evident and embryonic daughter sporocysts were present.

Mature mother sporocyst of *P. procyonis*

Recovered 16 days postinfection, 781 by 129. It already had liberated several daughter sporocysts and contained many other developing ones. Only evidence of eyespots was one small area of pigment granules in the body wall.

Daughter sporocysts of *P. procyonis*

Eight recovered 18 days postinfection. The largest (Fig. 3) was 2.64 mm by 0.11 mm. One snail had 25 daughter sporocysts but the individuals were smaller. The body is an elongated, muscular sac with a rounded posterior end, and thick-walled, bluntly pointed anterior end. A birth pore is anterior and subterminal. Each daughter sporocyst contained up to 40 cercariae.

Mature mother sporocyst of *P. adenocephala*

Recovered 33 days postinfection, 858 by 90. Many daughter sporocysts present in all stages of development, 15 liberated ones contained fully developed cercariae. Eyespots of mother sporocyst still present but fragmented.

Daughter sporocysts of *P. adenocephala*

Several recovered 33 days postinfection. Figure 9 shows characteristic form, this one being 1.43 mm by 0.13 mm. The body is an elongated, thin-walled sac, posterior end rounded, anterior end with thick-walled conical projection and subterminal birth pore. Distinctive shape of developing cercariae readily identifies this species. Each sporocyst contained 10–15 cercariae.

Cercariae

Cercariae of both species were obtained from laboratory reared *Menetus dilatatus buchanensis*. Cercarial emergence occurred 18–26 days postinfection, depending upon room temperatures and size of snail host. Most cercariae emerged during daylight hours. They swim tail first, rapidly undulating the tail stem. They swim to near the surface, cease swimming, and sink slowly, "head first," to the bottom.

Cercaria of *P. procyonis* (Figs. 5, 6)

Furcocercous, longifurcate, distomate, and pharyngeate. Twenty-five formalin fixed specimens were measured. Body 112–141 by 26–40; tail stem 180–262 by 29–51; furcae 156–235 long; acetabulum slightly postequatorial, nearly round, diameter 21. Anterior end of body spinose to level of intestinal bifurcation. Acetabulum covered with blunt spines arranged concentrically and pointing inward. Hairlike structures project laterally, one on each side of body at level of genital primordium. Numerous hairlike structures present on tail stem. Oral sucker subterminal averaging 33 by 21, prepharynx very short, pharynx 9 by 11; esophagus short, almost imperceptible; ceca terminating

at midlevel of acetabulum. Two pairs of unicellular, preacetabular, penetration glands; ducts opening separately. Excretory system, 2[(2 + 2 + 2) + (2 + 2) + (2)] with 10 pairs in body, 2 pairs in tail stem. Three ciliary patches in each main collecting tubule. Excretory bladder bipartite, tail stem duct bifurcates at furcae and terminates at pore at midlength on each furca.

Cercaria of *P. adenocephala* (Figs. 11, 12)

Furcocercous, longifurcate, distomate, and pharyngeate. Twenty-five formalin fixed specimens were measured. Body 134–187 by 31–44; tail stem 282–348 by 134–191; furcae 172–308 long; acetabulum slightly postequatorial nearly round, diameter 21. Anterior body spinose to level of intestinal bifurcation. Acetabulum covered with blunt, concentrically placed spines. Tail stem nearly cylindrical at junction with body, becoming much enlarged posteriorly, wider than body. Oral sucker subterminal averaging 38 by 22; prepharynx very short; pharynx 10 by 12, usually contiguous with oral sucker, esophagus short, ceca terminating at midlevel of acetabulum. Two pairs of unicellular penetration glands, 1 pair immediately preacetabular, the other pair just postacetabular; gland ducts open separately at oral sucker. Genital primordium located midway between acetabulum and posterior border of body. Excretory system, 2[(2 + 2 + 2) + (2 + 2) + (2)], 10 pairs in body and 2 pairs in tail stem. Excretory bladder bipartite in posterior body region; tail stem duct bifurcates at furcae, terminating at pore at midlength on each furca.

Comparison of Cercariae

The cercariae of both species are very similar in general morphology but differ in the arrangement of the penetration glands and the shape and size of the tail. The bulbous tail stem of *P. adenocephala* is the most striking difference. Its characteristic shape becomes evident during embryonic development in the daughter sporocyst and is not a result of osmotic swelling. The tail of *P. adenocephala* cercaria bursts shortly after mounting. Minor differences occur in the size of various organs in the two species.

Comments

P. procyonis Harkema, 1942 was found initially in the raccoon, *Procyon lotor* from North Carolina in 1939. Before its description was published, Chandler provided additional specimens from the raccoon in Texas. These were larger and mistakenly assumed to be mainly specimens older than those from North Carolina. Thus Harkema (1942) included both groups in his description.

Pharyngostomoides ovalis Chandler and Rausch, 1946, was described from the raccoon from Michigan and later was synonymized with *P. procyonis* by Dubois (1963), and is accepted. Our studies indicate that the large specimens found by Harkema and by Chandler were *P. adenocephala* as described in this paper.

Members of the genus *Pharyngostomoides* have been found only in the raccoon and it is probable that both species of parasites occur in this host throughout its range. Localities reported for *P. procyonis* are known, in some instances, to contain also *P. adenocephala*. *P. procyonis* has been reported from Texas and North Carolina (Harkema, 1942); Michigan (Chandler and Rausch, 1946); Virginia, North Carolina, South Carolina, Georgia, and Florida (Harkema and Miller, 1964). Although not demonstrated conclusively, we have reason to believe that both species of parasites are maternally transmitted (Harris, Harkema, & Miller, 1967).

Acknowledgments

Appreciation is expressed to Dr. Henry van der Schalie of the Museum of Zoology, University of Michigan, for the identification of snails.

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Metadena spectanda Travassos, Freitas, and Bührnheim, 1967 (Digenea: Cryptogonimidae) in Estuarine Fishes from the Gulf of Mexico[1]

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ABSTRACT: The first report of *Metadena spectanda* Travassos, Freitas, and Bührnheim, 1967, from North American waters is given. The cryptogonimid trematode was found in *Micropogon undulatus* and *Bairdiella chrysura* and redescribed from specimens from the former. Its similarity to some species of *Metadena* and to *Exorchis oviformis* Kobayashi, 1915, is discussed. *Exorchis* Kobayashi, 1915, is considered a synonym of *Metadena* Linton, 1910. *Metadena oviformis* is a new combination.

Metadena spectanda Travassos, Freitas, and Bührnheim, 1967, is one of eleven recognized species of *Metadena* Linton, 1910, not including *Paracryptogonimus leilae* (Nagaty, 1957) Manter, 1963, *P. apharei* (Yamaguti, 1953) Velasquez, 1961, *Neochasmus microvatus* (Tubangui, 1928) Tubangui and Masiluñgan, 1944, and *Siphoderina brotulae* Manter, 1934, which were all at one time considered species of *Metadena*. An additional species is being described by Robert Schroeder and was discussed briefly by Overstreet (1969). *Metadena spectanda* was previously known only from Brazil. It is redescribed below in order to add new information, provide a description that may be more readily available, and include ranges on the size of individuals that are commonly found in two estuarine sciaenid fishes of Mississippi and Louisiana.

Specimens were fixed in hot AFA and stained in Van Cleave's hematoxylin. Figures were drawn with the aid of a camera lucida, and measurements are given in microns.

Metadena spectanda Travassos, Freitas, and Bührnheim, 1967

HOSTS: *Micropogon undulatus* (Linnaeus), Atlantic croaker; *Bairdiella chrysura* Lacépède), silver perch.

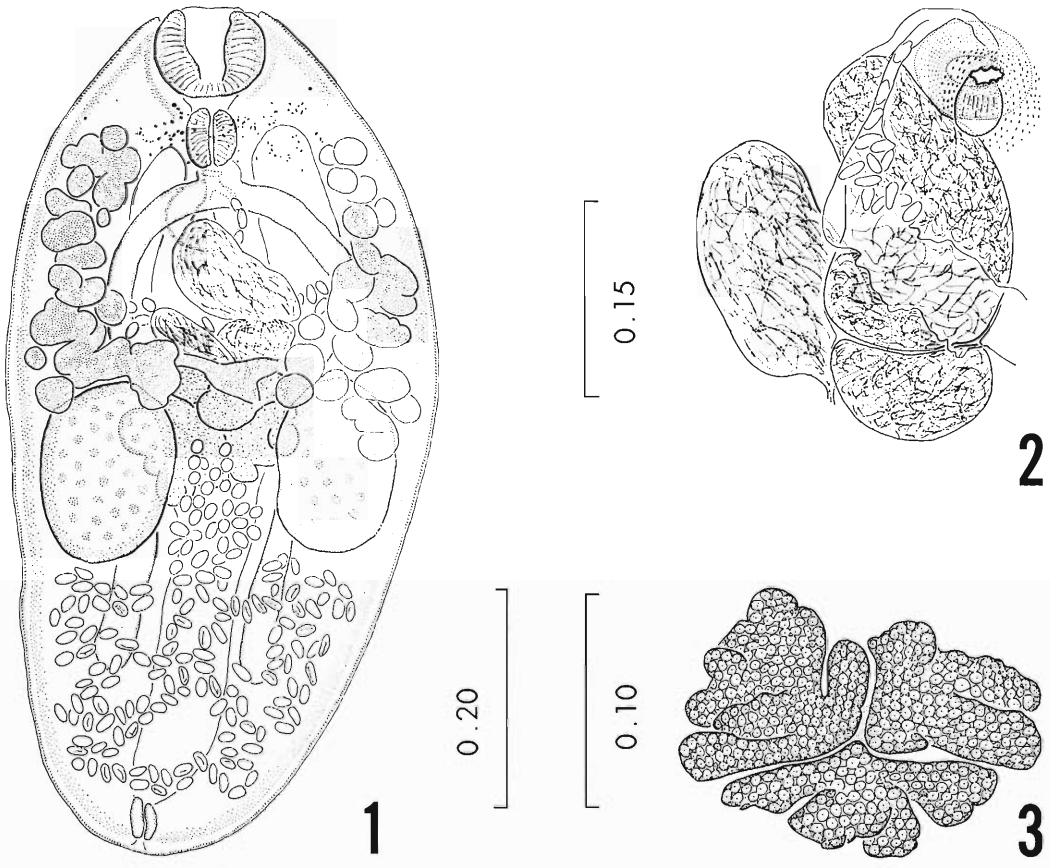
SITES: Intestine and pyloric ceca.

LOCALITIES: Mississippi and Louisiana waters along coast of Gulf of Mexico.

SPECIMEN DEPOSITED: USNM Helm. Coll. No. 71765.

DESCRIPTION (based on 21 mature specimens from *Micropogon undulatus*): Length 320–1,216; width 209–612 or 43–68% of body

[1] This study was conducted in cooperation with the Department of Commerce, NOAA, National Marine Fisheries Service, under Public Law 88-309, Project 2-85-R.



Figures 1–3. *Metadena spectanda.* **1. Wholemount, dorsal view. 2. Terminal genitalia and seminal receptacle, ventral view. 3. Ovary, ventral view. Scale values are millimeters.**

length; covered with minute spines. Eyespot pigment usually dispersed, near pharyngeal level. Cephalic glandular cells conspicuous in forebody (not illustrated). Oral sucker retractile into anterior end of body, without trace of oral spines, 70–133 long by 79–149 wide. Acetabulum conspicuous, 35–77 by 37–91. Sucker width ratio 1:0.34–0.69, range wide primarily because of variation in acetabular width. Forebody 84–226 long, 19–32% of body length. Prepharynx usually less than ½ length of pharynx or occasionally longer. Intestinal bifurcation at or near acetabular level; ceca usually ventral to but occasionally immediately medial to testes, terminating about halfway between testes and posterior end of body.

Testes longer than wide, symmetrical, well separated; left testis 56–219 long by 38–128 wide; right testis 74–222 by 49–119. Posttesticular space 74–468 long, 20–43% of body length. Seminal vesicle elongate, bipartite but not always discernable in fixed specimens, either dorsal, lateral, or posterior to acetabulum. Genital atrium either longer or shorter than depth of acetabulum; genital pore median and immediately anterior or ventral to acetabulum.

Ovary at midbody, ventral to and between anterior borders of testes, occasionally not reaching one of testes; multilobed, some individuals with 3 primary lobes, each lobe with 3–6 secondary lobes, other individuals with numerous lobes but no discernable primary lobes; occupying space 77–198 long by 95–285 wide.

Vitelline follicles in lateral fields between levels of oral sucker or pharynx and testes, confluent dorsal to anterior portion of ovary. Seminal receptacle near and often larger than seminal vesicle. Laurer's canal not observed. Uterus filling most of body posterior to gonads and also that between testes and level of posterior border of acetabulum. Eggs 19–28 long by 10–16 wide in mounted specimens, 23–30 by 12–15 in living ones; operculated shell having granular appearance.

Excretory vesicle bifurcating at or near ovarian level; arms extending to pharyngeal level; pore terminal.

Discussion: Two paratypes (30-152 b and c) were kindly loaned to me by the late Dr. J. F. Teixeira de Freitas for examination. I did not see the originally described cirrus sac in either specimen, a character which, in the true sense, would place the species into another genus and differentiate it from my specimens. My specimens and the loaned ones both have prominent eyespot pigment granules and granular appearing egg-shells. The only apparent difference between the North and South American specimens is the average size. The length of the paratypes from *Paralichthys brasiliensis* (Ranzani) were listed as 0.73 to 1.45 mm, and those from *Lutjanus jocu* (Bloch and Schneider), including the holotype, as 1.81 to 2.00 mm (Travassos, Freitas, and Bührnheim, 1967), whereas the length of those in my collection from sciaenid fishes range from 0.32 to 1.22 mm. Only a few of my specimens, including numerous worms not used for the redescription, overlap in size with any of those from *P. brasiliensis*. My specimens from *Bairdiella chrysura* agree in all respects with those from *Micropogon undulatus*.

The vitellaria in *M. spectanda* are in the shoulder region as they are in *M. lopastoma* Winter, 1958, *M. magdalenae* Arai, 1963, *M. pauli* (Vlasenko, 1931), and *M. eurystoma* Oshmarin, 1965. The first two of these species have relatively large oral suckers and vitelline follicles which transverse the bodies. The last two show more similarity to *M. spectanda* and are also reported from sciaenid fishes. Variations in the last two species are not well known, the latter being described from a single specimen. *Metadena pauli* is rounder than *M. spectanda*, with distinctly extratesticular ceca. In *M. eurystoma* the oral sucker is broad at the posterior portion, and the testes are wider than long. Additional examination of material of these two species may reveal that not all species of *Metadena* are valid.

Metadena spectanda may differ from all the other species by having a bipartite seminal vesicle and testes that are occasionally in an extracecal location. The external seminal vesicle described for *M. eurystoma* by Oshmarin (1965) is probably a seminal receptacle. The testes illustrated by Janiszewska (1953) for *M. depressa* (Stossich, 1883) are nearly extracecal.

In view of the bipartite seminal vesicle in *M. spectanda*, the location of the vitellaria and ceca in several species, and the small unlobed ovary in *M. lutiani* (Yamaguti, 1942), I place *Exorchis oviformis* Kobayashi, 1915, as a synonym of *Metadena oviformis* (Kobayashi, 1915) comb. n. and consequently *Exorchis* Kobayashi, 1915, as a synonym of *Metadena* Linton, 1910. The only feature that could be used to separate the species of *Exorchis* from those of *Metadena* is the apparent consistently extracecal location of the testes in the former, and I do not consider that of generic magnitude.

Acknowledgments

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Cercaria amblemae sp. n., a Rhopalocercous Cercaria from *Amblema plicata* (Say)

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ABSTRACT: A new species of rhopalocercous cercaria, *Cercaria amblemae*, from the bivalve *Amblema plicata* (Say) is described. The new species is distinguished by the number of sensory papillae, number of penetration glands, and the presence of a neck-like appendage of the transformed tail.

During the late summer and autumn of 1969 a survey was made of the metazoan parasites of unionid clams in Hickory Creek arm of Garza-Little Elm Reservoir at Sycamore Bend Park, Denton County, Texas. Eight of 146 specimens of *Amblema plicata* (Say) collected were infected with sporocysts producing a rhopalocercous cercaria of the trematode family Gorgoderidae, subfamily Phyllodistomatinae. The entire cercaria was drawn to scale from measurements of representative specimens allowed to emerge naturally *in vitro*, fixed in A.F.A., slowly glycerinated, and mounted in glycerine jelly. Details were added freehand from the study of living organisms and whole mounts stained with Harris' hematoxylin, Ehrlich's hematoxylin, or precipitated borax carmine. Live specimens were found to be most desirable for visualizing certain structures, especially papillae, penetration glands, and the excretory system. Metacercariae were drawn from glycerinated whole mounts with the aid of a Leitz drawing attachment. All measurements are in microns unless otherwise indicated.

Description of Stages

Cercaria amblemae sp. n. (Fig. 2)

DESCRIPTION: Distomate, rhopalocercous cercaria. Withdraws into tail immediately upon emergence from host. Body subcylindrical preacetabularly, flattened postacetabularly; length 557–821; maximum width 93–143 at level just posterior to acetabulum. Tail subcylindrical and corrugate; length 278–493 (almost ⅔ of body length); maximum width 64–121; broadly attached to body. Tegument of body thin, even; without spines; papillated. Four pairs of papillae on ventral surface of body anterior to acetabulum and 6 pairs posterior to acetabulum. Dorsal surface with 6 pairs of anterior and 6 pairs of posterior papillae. Lateral marginal papillae, 18 or 19 pairs, the last 2 pairs of which are setate. In cercariae having lost their tails an additional 3 dorsal and 3 ventral papillae on margins of terminal posterior concavity surrounding excretory papule. Oral sucker 70–87 long, 71–86 wide, mouth subterminal. Thirty papillae distributed over oral sucker, six of which are in oral opening. Ventral sucker 71–107 in diameter, slightly larger than oral sucker; 207–357 from anterior end of body, 228–414 from posterior end; bearing 16 or 17 papillae. Esophagus short, bifurcation anterior to midpoint between suckers; ceca extend to near end of body. Penetration glands 11 per side, opening onto dorsal surface above oral sucker. Genital primordia well-developed. Ovary postacetabular, slightly overlapping cecum; amphitypy not observed; vitellaria compact, immediately postacetabular, rarely extending beyond lateral margins of ceca. Testes post-ovarian, between or slightly overlapping ceca. Uterine primordium and short portion of vas deferens joining preacetabular genital atrium. Excretory bladder tubular; primary collecting ducts extending anteriorly and reflexing at level of cecal bifurcation, secondary ducts joining primary duct posterior to acetabulum; 32 pairs of flame cells (distribution shown in Fig. 2d).

HOST: *Amblema plicata* (Say).

SITE OF INFECTION: Gonad and digestive gland.

LOCALITY: Garza-Little Elm Reservoir, Denton County, Texas.

TYPE SPECIMEN: U.S.N.M. Helm. Coll. No. 71426.

REMARKS: Rhopalocercous cercariae are a unique group characterized by a club-like, corrugated tail usually at least as wide as the

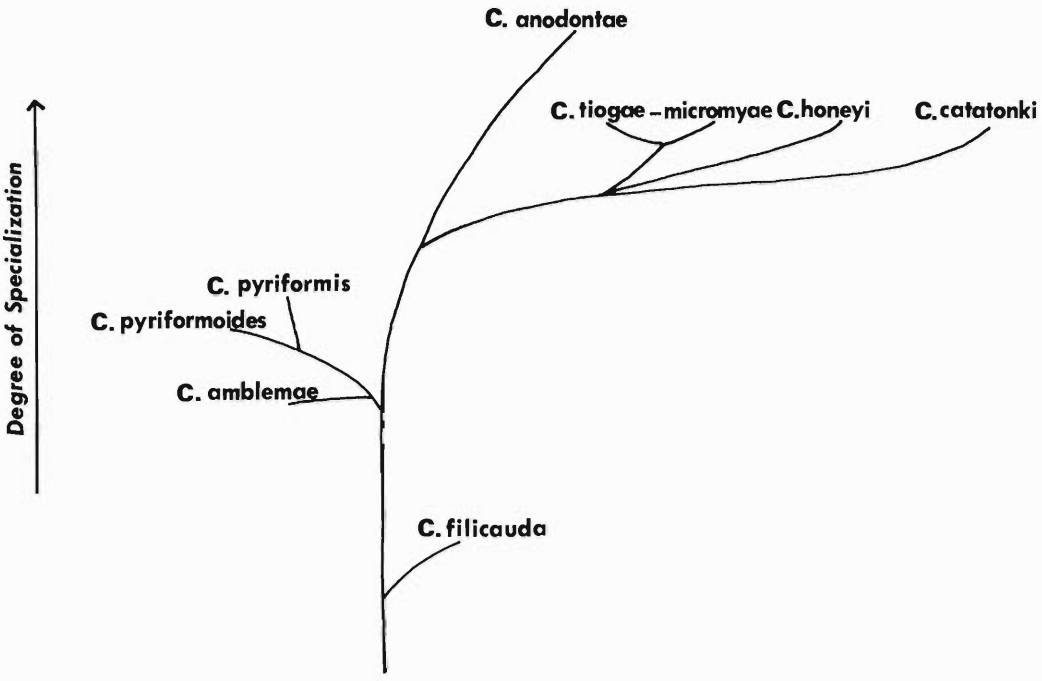


Figure 1. Suggested relationships among rhopalocercous cercariae.

body. This tail is capable of expansion to form a balloon-like sac within which metacercarial encystment occurs. These cercariae typically are astylate, apharyngeate, and possess markedly well-developed genital primordia. Eight rhopalocercous species have previously been described for North America.

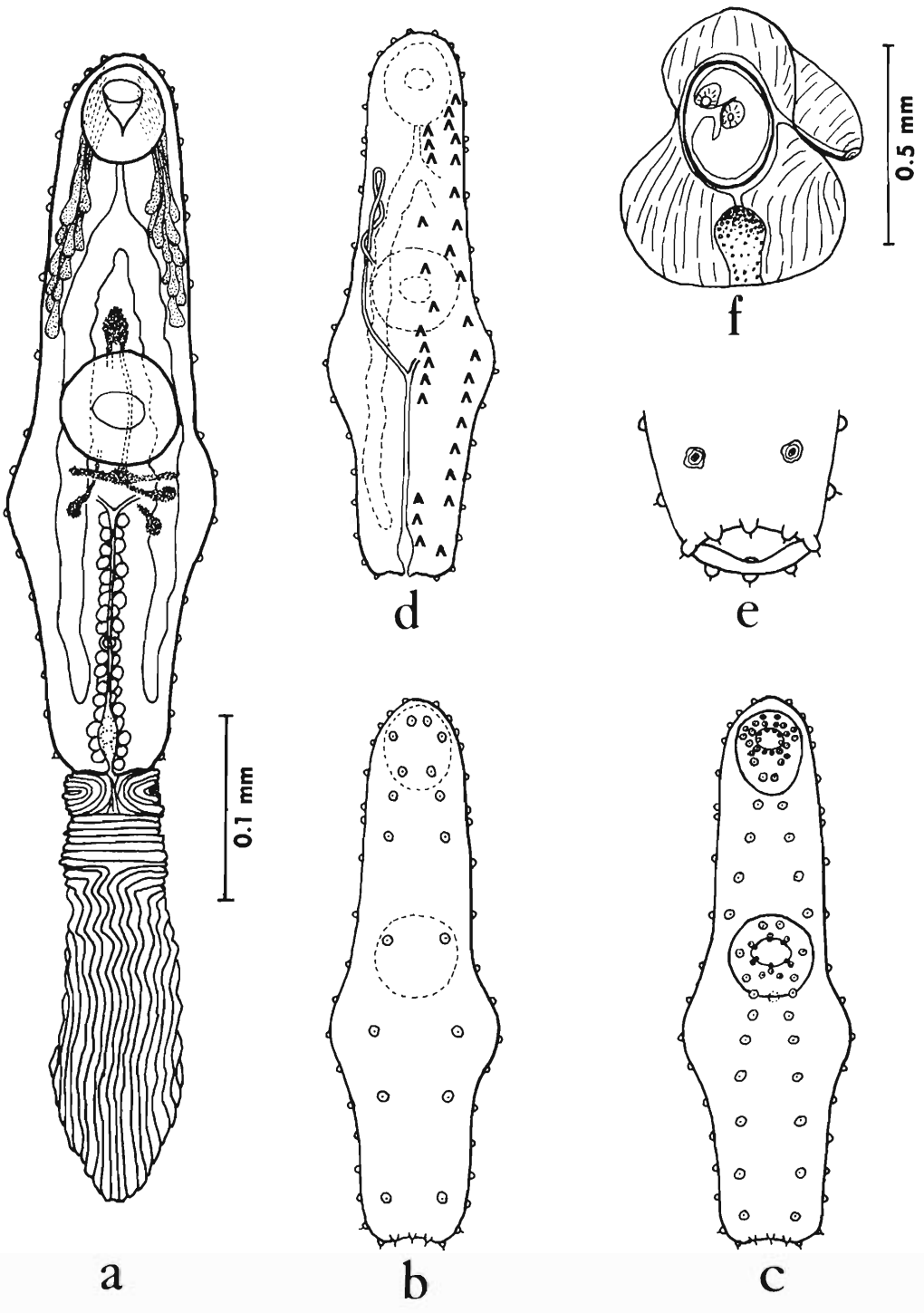
Cercaria amblemae sp. n. bears similarities to both *C. pyriformis* Fischthal, 1951 and *C. pyriformoides* Coil, 1954. However, *C. amblemae* differs from *C. pyriformis* in number of oral papillae (30 in *C. amblemae*, 33 in *C. pyriformis*), in number of anterior ventral papillae (8 in *C. amblemae*, 12 in *C. pyriformis*), in number of posterior ventral papillae (12 in *C. amblemae*, 10 in *C. pyriformis*), and in the absence in *C. pyriformis* of the neck-like appendage of the transformed tail. This neck is also lacking in *C. pyriformoides*. In addition, *C. amblemae* can be distinguished from *C. pyriformoides* by the number of oral papillae (30 in *C. amblemae*, 6 of which are in the mouth, and 34 in *C. pyriformoides*, 8 of which are in the mouth), by number of posterolateral papillae bearing setae (last 2 pairs in *C. amblemae*, last 3 pairs in *C. pyriformoides*), by number of anterolateral marginal papillae (18 in *C. amblemae*, 16 in *C. pyriformoides*), and by number of posterior ventral papillae (12 in *C. amblemae*, 14 in *C. pyriformoides*).

Daughter Sporocyst

DESCRIPTION: Elongate ellipsoid, but more acutely tapered at one end. Cercarial birth pore subterminal at narrow end, distinguishable by lip-like process of sporocyst wall. Ma-

→

Figure 2. *Cercaria amblemae* sp. n. from *Amblema plicata* (Say). a. Cercaria whole mount, ventral view. b. Distribution of dorsal papillae. c. Distribution of ventral papillae. d. Excretory system, showing distribution of flame cells. e. Arrangement of setate papillae on posterior margin. f. Metacercaria within transformed tail.



ture specimens 520–960 long by 200–580 wide. Usually contains approximately 2 active cercariae, 1 embryonic cercaria, and 1 or more germ balls; numerous free cells also present.

REMARKS: Living specimens of *C. amblemae* were studied in order to determine the mode of exit of the cercaria from the sporocyst. The mature cercaria is very active and rotates frequently within the sporocyst. The wall of the sporocyst is drawn into the oral sucker, the worm pulling repeatedly in this way at the lining. This activity is carried on at both ends of the sporocyst, but the effort seems to be most concentrated at the narrow end. The result appears to be an erosion of the wall so that eventually the worm succeeds in thrusting its way out, usually at the birth pore, where the cell layer is apparently weakest. Emergence *in vitro* was promoted by lowering the osmotic pressure of the external environment of the sporocyst.

No attempt was made to determine the role of the penetration glands in this process; however, it should be noted that Fischthal (1951) stated that one pair of these glands is apparently utilized in emergence from the host. Eleven pairs of penetration glands were counted in specimens of *C. amblemae* which had emerged naturally from sporocysts *in vitro*.

Metacercaria
(Fig. 2f)

DESCRIPTION: Metacercarial encystment occurs within transformed cercarial tail. Secreted cyst diameter (at about 12 hours) 287. Main portion of transformed tail (containing cyst chamber) pyriform with a broad, laterally directed, neck-like appendage at anterior end. Metacercaria (at 12 hours) differs from cercaria principally in reduction of number of cystogenous glands.

REMARKS: Under natural conditions the process of encystment begins as soon as the cercaria emerges from the host tissues into the mantle cavity. In the laboratory the metacercariae of *C. amblemae* were expelled through the excurrent siphons of isolated hosts. Observations on the mechanism of the encystment process verify the conclusions of Parker (1932) and Fischthal (1951) that tail transformation is an osmotic phenomenon. Cercariae maintained in clam plasma underwent no transformation. However, encystment was easily induced by dilution of the tissue fluid with distilled water. Obviously, in nature this necessary decrease in environmental osmotic pressure would be encountered upon emergence into the water-filled mantle cavity of the host.

Subsequent events of tail transformation in *C. amblemae* conform to Fischthal's (1951) description. As water is absorbed, the cuticle of the proximal transversely corrugated region begins to separate from the underlying cellular layer and to balloon outward and upward around the posterior end of the worm. Coincident with cuticular disjunction is a progressive contraction of the inner cellular layer toward the posterior, so that as the cuticle of the tail expands, the attached worm extends maximally, thereby narrowing its width to facilitate passage through the canal being formed by simultaneous invagination of the cuticle and cell layer contraction. In many instances movements of the worm during envelopment were noted to result in a premature separation from the tail, so that encystment could not be completed successfully.

As the transformation is completed, the worm normally breaks the fragile attachment to the tail cuticle and contracts into the central region of the now pyriform tail. The cercarial chamber is lined with the invaginated cuticle seen in the untransformed tail as the more finely rugose, thin cuticle of the anterior one-half of the transversely corrugated region. This chamber is continuous with the exterior through a constricted canal lined with cuticle of the same origin. Posteriorly the cuticle of the chamber remains attached to the contracted cell mass. The neck of the transformed tail is derived from the thicker, more deeply rugose cuticle of the posterior one-half of the transversely corrugated region of the untransformed tail. The pyriform outer wall arises from the longitudinally corrugated portion of the tail.

As soon as the worm has retracted into the central chamber of the tail, it begins a series of rotational movements interspersed with thrusts of both anterior and posterior ends. As these movements begin, cystogenous material appears as a deposition of hyaline material against the chamber wall. This layer displays considerable elasticity in withstanding the active probings of the trematode. This cystogenous secretion is evidently contributed pri-

marily by the hyaline secretory cells surrounding the bladder, as has been proposed by other workers. Coil (1954a) found that these cells in the rhopalocercous species he studied disappeared within 24 hr after tail transformation. Metacercariae of *C. amblemae* fixed 12 to 18 hr after initiation of encystment still retained at least ½ to ⅔ of the cystogenous glands. Those having disappeared were the ones nearest the posterior extremity of the bladder, suggesting that these cells discharge in an orderly fashion from posterior to anterior.

Discussion

The most obvious characteristic common to all rhopalocercariae is the unique tail. In *C. amblemae*, as in all previously described rhopalocercous forms, the tail in cross-section consists of two distinct layers, the outer corrugated cuticle and beneath and only loosely associated with it a layer of cells with large, dense nuclei. The attachment of tail to body is very fragile, and quite often the tail is lost before, during, or after emergence from the sporocyst. It was not possible to determine with certainty the exact nature of this attachment in *C. amblemae*. However, in certain whole mounts it appeared that continuity between body and tail occurred only in the cuticular layer. The inner cellular layer of the tail appeared to close across the central cavity at the anterior end to form a concave surface with flared margins. This concave surface was distinctly separated from the posterior end of the body. This space has also been noted in other rhopalocercariae by Fischthal (1951). Since the terminal concavity around the excretory pore of the body proper can be opened and closed to a considerable extent by muscular contraction, it seems possible that the margin of this concavity could, during activity, be constricted so as to grasp around the flared anterior surface of the cellular layer of the tail. This would achieve a firmer attachment than that provided by the cuticle alone. This possible explanation of tail attachment is consistent with observations on the process of tail transformation and metacercarial encystment.

The numerous papillae distributed over the body surfaces and the suckers of *C. amblemae* are characteristic of all rhopalocercariae and have been noted in many other gorgoderid larvae. Four types of papilla are present in *C. amblemae*. The most common form is somewhat irregular in outline and within it are visible two concentric rings. In the center of the innermost ring is a refractile granule. A second, less common, type of papilla contains a distinctly larger, denser, greenish granule. Three pairs of papillae are located on the inner margin of the elliptical mouth aperture—two pairs on the anterior border and one papilla in either corner. The second of these papillae from the midline on either side is of the less common type bearing the larger, dense granule (noted also in other species by Fischthal, 1951). The third type of papilla is double, seemingly a combination of the two previously described types. Six of these double papillae are distributed around the margin of the acetabular orifice with the component bearing the larger granule nearest the sucker opening. A fourth type of papilla, bearing a single seta, is found only on the posterior extremity of the body. The excretory pore opens through a papule centered in a terminal concavity. On the outer margin of this concavity, along the line of tail attachment are distributed 8 of these setate papillae. Setae are also found on the last pair (or 2 pairs) of posterolateral marginal papillae. (It should be noted for clarity that in flattened, extended whole mounts one pair of setate papillae on the margin of the terminal concavity usually appears to be in series with the posterolateral marginal papillae, thereby giving the appearance of there being 2 pairs of setate posterolateral marginal papillae, 3 dorsal terminal papillae, and 3 ventral terminal papillae.)

The double papillae of the acetabulum are present in all rhopalocercous forms described by both Fischthal (1951, 1954) and Coil (1954a), as well as in certain other gorgoderid cercariae. However, setate posterior papillae have been described previously only for *C. pyriformoides* and *C. anodontae* (Coil, 1954a). Five setate papillae in the vicinity of the excretory pore were figured by Coil for *C. pyriformoides* but were not mentioned in the text of the description. Their exact placement was not indicated. The total number of setate papillae is, however, the same in both *C. amblemae* and *C. pyriformoides*, despite seeming differences in location. Since these papillae are difficult to resolve microscopically and to visualize in spatial perspective, the apparent discrepancy between descriptions of these

papillae probably lies with the interpretations only.

In most aspects the rhopalocercarial group is quite uniform, and traits of diagnostic value are limited. Distinctions have been based in most cases upon number of penetration glands and number and placement of papillae. Number and arrangement of flame cells are of potential systematic importance, but at present flame cell formulae have been determined only for *C. pyriformoides* and *C. micromyae* (Fischthal, 1951). Total flame cell count was ascertained for *C. amblemae*, but the arrangement of the collecting ducts could not be distinguished.

With the addition of *C. amblemae* to the list of known rhopalocercariae, it has become apparent that the form of the tail after transformation to the metacercarial stage may serve as a diagnostic characteristic in this group. These cercariae fall into 4 categories based on this criterion: (1) Transformed tail with sticky posterior filament (*C. filicauda* Fischthal, 1951), (2) Transformed tail pyriform, with an anterior, laterally directed neck (*C. amblemae*), (3) Transformed tail pyriform, without anterior neck-like appendage (*C. pyriformoides*, *C. pyriformis*), and (4) Transformed tail ovoid, without appendages (*C. anodontae*, *C. catatonki* Fischthal, 1951; *C. honeyi* Fischthal, 1951; *C. micromyae*, and *C. tiogae* Fischthal, 1954).

It should be noted that there are certain certain important limitations in the use of sensory papillae as taxonomic criteria for the rhopalocercaria. Although within a species papilla number and location are constant for the oral sucker and the preacetabular dorsal and ventral surfaces, considerable variation is often seen on the postacetabular ventral surface and to a lesser extent on the postacetabular dorsum. In at least 4 species the number of papillae on the posterior margin of the acetabulum has been noted to vary slightly (by 1) among conspecific individuals.

Although the number of posterior dorsal papillae has never been noted in any rhopalocercous species to vary by more than one, those of the posterior ventral surface in *C. catatonki* range in number from 9 to 14, in *C. micromyae* from 11 to 15, and in *C. tiogae* from 10 to 13. Obviously, therefore, distinction between taxa on the basis of posterior papillae is highly inadvisable. It is surprising, therefore, to note that Fischthal (1954) described *C. tiogae* as being distinct from *C. micromyae* only in papilla counts in these three areas of variation: posterior margin of acetabulum—2 or 3 in *C. tiogae*, 2 (rarely 3) in *C. micromyae*; posterior dorsal surface—8 (or 9) in *C. tiogae*, 7 (or 8) in *C. micromyae*; posterior ventral surface—11 (12, 10, or 13) in *C. tiogae*, 13 (11, 12, 14, or 15) in *C. micromyae*. The marked degree of overlap in papilla count between these forms together with the fact that they are essentially identical in all other significant characteristics except host, suggests that *Cercaria tiogae* (Fischthal, 1954), should probably be synonymized with *C. micromyae* (Fischthal, 1951). (*C. tiogae* was reported from *Alasmidonta varicosa* [Lamarck] in Tioga County, New York; *C. micromyae*, from *Alasmidonta marginata* [Say] in the Huron River, Michigan.)

Despite the status of *Cercaria rhyticerca* Parker, 1932, as a *nomen dubium*, many of the characteristics given for the organism are of interest. This form was indicated to have the general body and tail conformation typical of all rhopalocercariae. The reproductive primordia were typically rhopalocercarial. The excretory system was elucidated from bladder through anterior and posterior main collecting ducts. The common collecting tubule on either side was noted to run forward well anterior to the cecal bifurcation before reflexing posteriorly. Division into the main collecting ducts occurred just posterior to the acetabulum. This pattern is quite similar to that described by Coil (1954a) for *C. pyriformoides*. The ridges and furrows of the body described by Parker (1932) were undoubtedly artifacts of clearing and mounting. Such deformations were found to be difficult to avoid in preparation of *C. amblemae*.

Of primary significance here is the fact that *C. rhyticerca* was shown to have a transformed tail identical to that of *C. amblemae*. These two species are the only forms described in which the transformed tail is pyriform with a neck-like anterior appendage. *C. rhyticerca* was hosted by *Amblema costata*, which is considered by many authorities to be but an ecophenotype of *A. plicata*, the host of *C. amblemae*. The principal deficiency of Parker's description is lack of detail on number of penetration glands and on number and placement

of papillae, so that the phyletic affinities of *C. rhyticerca* cannot be established with certainty. However, the available information suggests a very close relationship to *C. amblemae*. The only notable differences between the two forms seem to be the level of reflexion of the common collecting duct (at the level of cecal bifurcation in *C. amblemae*, anterior to bifurcation in *C. rhyticerca*) and the width of the tail (slightly less than body width at acetabular level in *C. amblemae*, only about ⅓ of body width in *C. rhyticerca*).

Attempts to show evolutionary relationships of rhopalocercariae within the group itself and with other types of gorgoderid cercariae have been made by Fischthal (1951) and Coil (1954b). Such schemes are, of course, only tentative since the adults are unknown for most of these larvae and since many cercarial characteristics are probably coenogenetic and adaptive for specific life cycles. Both Fischthal (1951) and Coil (1954b) have paralleled the apparent lines of evolution of the gorgoderid cercariae with those of their molluscan hosts. As a result, the Fischthal–Coil scheme shows three main branches of cercarial phylogeny, following the evolution of the pelecypod families Unionidae, Sphaeriidae, and Dreissenidae. The rhopalocercariae are limited completely to unionid clams, the oldest of the three freshwater bivalve families. Macrocercous forms are mainly parasites of the Sphaeriidae, whereas the only known microcercous form is from the Dreissenidae. Coil (1954b) has described two apparently primitive cercariae from unionids, characterized by stylets and slender natatory tails, which are probably close to the common ancestral line hypothesized for all gorgoderid cercariae.

Relationships within the rhopalocercous group are even less clear because of the relative uniformity among these organisms. Both Fischthal (1951) and Coil (1954b) have concluded that there is a trend in the group toward reduction in size of the tail. Under this assumption *C. filicauda* with its filamentous tail appendage is the most primitive rhopalocercaria known. Since *C. filicauda* possesses 11 pairs of penetration glands, the maximum number seen in the group, Coil assumed a concomitant reduction in number of these structures. He also suggested a trend toward smaller body size. With these characteristics as the basic criteria for analysis, the rhopalocercous cercariae can be divided into 4 groups. The first group contains only the unique *C. filicauda*. The second group possesses the primitive characteristics of 11 pairs of penetration glands, large body size, and a tail which becomes pyriform upon transformation (*C. amblemae*, *C. pyriformoides*, and *C. pyriformis*). The third group is characterized by 9 pairs of penetration glands, intermediate body size, and a relatively simple, ovoid transformed tail (*C. micromyae*, *C. tiogae*, *C. honeyi*, and *C. catatonki*). The fourth and most highly specialized group, including only *C. anodontae*, possesses only 7 pairs of penetration glands, is the smallest in size, and shows the simple ovoid type of transformed tail. These phenotypic relationships are depicted diagrammatically in Figure 1.

Evaluation of *Cercaria amblemae* in terms of the supposed trends in rhopalocercarial evolution suggests that it is one of the most primitive of the known members of this group. In body size it is second only to *C. pyriformoides*. Its tail to body length ratio (approximately 2:3) is greater than that of any other rhopalocercarial species (ratios approximately 1:3–1:2). The seemingly functionless neck-like appendage of the transformed tail of *C. amblemae* appears to be an excess that has been eliminated in all more advanced species.

Key to Rhopalocercariae of North America

1. a. Transformed tail with long posterior filament ..... *Cercaria filicauda* Fischthal, 1951
   b. Transformed tail without posterior filament 2
2. a. Eleven of penetration glands, transformed tail pyriform 3
   b. Less than 11 pairs of penetration glands, transformed tail ovoid 5
3. a. Four pairs of ventral papillae anterior to acetabulum 4
   b. Six pairs of ventral papillae anterior to acetabulum ..... *Cercaria pyriformis* Fischthal, 1951
4. a. Thirty oral papillae (6 in mouth), transformed tail with laterally directed anterior neck ..... *Cercaria amblemae* sp. n.

b. Thirty-four oral papillae (8 in mouth), transformed tail without neck ..... *Cercaria pyriformoides* Coil, 1954

5. a. Nine pairs of penetration glands, 15–17 acetabular papillae 6
   b. Seven pairs of penetration glands, 13 acetabular papillae ..... *Cercaria anodontae* Coil, 1954

6. a. One papilla on posterior acetabular margin 7
   b. Two (or 3) papillae on posterior acetabular margin 8

7. a. Five dorsal papillae posterior to acetabulum ..... *Cercaria catatonki* Fischthal, 1951
   b. Seven (or 8) dorsal papillae posterior to acetabulum ..... *Cercaria honeyi* Fischthal, 1951

8. a. Seven (sometimes 8) dorsal papillae posterior to acetabulum ..... *Cercaria micromyae* Fischthal, 1951
   b. Eight (rarely 9) dorsal papillae posterior to acetabulum ..... *Cercaria tiogae* Fischthal, 1953

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Report of the Brayton H. Ransom Memorial Trust Fund

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The Development of the Endogenous Stages of *Eimeria ninakohlyakimovae* (Yakimoff and Rastegaieff, 1930) in Domestic Sheep[1]

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ABSTRACT: Forty-five mixed-breed lambs, 1 to 4 months of age, were used to study the endogenous stages in the life cycle of *Eimeria ninakohlyakimovae*. The experimentally infected lambs were killed at daily intervals from 1 through 14 days after inoculation. Sections of intestinal tissue were prepared by routine methods for histological examination. Two generations of schizonts were seen. Mature, first-generation schizonts, first seen 9 days after inoculation, had an average diameter of about 290 μ and many thousands of merozoites, averaging 11.9 by 2.1 μ. These macroscopic schizonts were most numerous 1.5 to 4.5 m anterior to the ileocecal valve. Young, first-generation trophozoites, first observed 3 days after inoculation, occurred in cells of the lamina propria adjacent to the base of the intestinal crypts. Cells harboring first-generation schizonts underwent an increase in volume of cytoplasm, nucleus and nucleolus, and each such host cell was surrounded by an envelope of flattened cells. In immature schizonts, a peripheral layer of nuclei underwent a series of infoldings, giving rise to spheroidal blastophores. Merozoites appeared as outgrowths from the blastophores. Many mature first-generation schizonts, first seen 9 days after inoculation, were invaded by leucocytic cells, and the merozoites were phagocytized by macrophages. Second-generation schizonts and sexual stages occurred in epithelial cells lining the crypts in the large intestine. Mature schizonts, observed 10 to 11 days after inoculation, had a mean diameter of about 12 μ and a mean of 24 merozoites, with mean dimensions of 5.5 by 1.4 μ. The schizonts developed in 1 to 2 days and the merozoites formed in a manner similar to that in individual blastophores of first-generation schizonts. Sexual stages occurred 11 through 14 days after inoculation. In mature microgametocytes, which averaged 15.0 by 11.6 μ, the microgametes were arranged peripherally about a central residual mass. The mean size of mature macrogametes and oocysts was 16.1 by 12.3 μ and 17.6 by 13.3 μ, respectively. The prepatent period in 4 lambs was 11 days and the patent period in 2 lambs was 7 days.

Eimeria ninakohlyakimovae Yakimoff and Rastegaieff, 1930, is one of the most pathogenic of sheep coccidia (Levine and Ivens, 1970). Some information as to the endogenous development of *E. ninakohlyakimovae* in domestic sheep has been reported by Lotze (1954) and by Hammond, Kuta, and Miner (1967). Reports of the endogenous stages in goats (Balozet, 1932; Sayin, 1964) differ in some respects from those in sheep. The present study was undertaken in order to obtain more detailed information concerning the endogenous development of this parasite in domestic sheep.

Material and Methods

Forty-five lambs, 1 to 4 months old, each a mixture of Columbian, Rambouillet, Hampshire, and Suffolk breeds, were used. Twenty-eight lambs, 1 week or less in age, were obtained during the springs of 1967, 1968, and 1969. These lambs were each kept with their ewes in individual pens throughout the experiment; they were inoculated when they were 3 to 4 weeks old. Seventeen lambs, 3 to 4 months old, obtained during the falls of 1967 and 1968, were maintained individually in pens without their ewes. Each pen was about 1.2 by 2.5 m in size, partially covered with a sloping roof, and had a dirt or crushed rock floor, covered with straw, which was replaced twice weekly. All sheep had daily access to dry alfalfa, mixed grain, and water.

The inoculum used consisted only of *E. ninakohlyakimovae* oocysts obtained from experimentally-infected lambs. Two lambs were each inoculated *per os* with 5,000 to 10,000 oocysts of *E. ninakohlyakimovae* to determine the prepatent and patent periods of infection and to obtain additional inoculum. These lambs were later reinoculated with higher dosages of oocysts for study of the endogenous stages.

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Trophozoites of first-generation schizonts were obtained by introducing sporozoites into intestinal fistulas. These were prepared in each of two 4-month-old lambs by methods described earlier (Chobotar, Hammond, and Miner, 1969), except that the segment isolated was 3 m anterior to the ileocecal valve. Five days after surgery, the sporozoites excysted by the methods of Hibbert and Hammond (1968) from 5,000,000 oocysts were introduced with a pipette into each fistula. The two lambs having fistulas were killed 2½ and 3 days after inoculation, respectively, and tissues were prepared for study as described below.

Trophozoites of second-generation schizonts were obtained by introducing merozoites into a ligated cecum in each of two 4-month-old lambs, by methods already described (Hammond, Anderson, and Miner, 1963). Fifty million first-generation merozoites were introduced with a syringe into each cecum. These merozoites were obtained from mature first-generation schizonts dissected out of the intestinal tissue of 2 lambs inoculated 10 days earlier with oocysts. The interval between the killing of the lambs used to provide merozoites and the introduction of these merozoites into the ligated ceca was 1½ to 2 hr. One cecal biopsy 36 hr after inoculation and 2 biopsies 24 and 48 hr after inoculation were performed in the 2 lambs, respectively. Biopsies performed before inoculation in both lambs were used as controls.

The remaining endogenous stages of the parasite were obtained from 39 lambs, each of which had been inoculated *per os* with 50,000 to 1,000,000 oocysts, and from the 2 lambs used as a source of merozoites for introduction into ceca. Fecal samples were collected intermittently from uninoculated lambs and daily from inoculated lambs. The samples were examined for oocysts by a modified McMaster technique (Whitlock, 1948). Lambs were killed at daily intervals ranging from 1 day after inoculation through 14 days. Sections of tissue from the abomasum, cecum, upper colon, middle colon, lower colon, and from the small intestine at 1.5 m intervals anterior to the ileocecal valve were fixed in Zenker's fluid; all tissue samples were sectioned in paraffin, and stained with hematoxylin—eosin (H and E) or iron hematoxylin. Intestinal tissues were also prepared according to the method of Feulgen (Barka and Anderson, 1963) and of Himes and Moriber (1956). Merozoites were obtained for study from living first-generation schizonts dissected from the lamina propria of lambs harboring 10- to 12-day experimental infections. Living and fixed first-generation merozoites were prepared for study and observed by the methods of Hammond, Ernst, and Goldman (1965).

Drawings were made with the aid of a camera lucida and photographs with the aid of a Zeiss photomicroscope. Living specimens and those in permanent preparations fixed in

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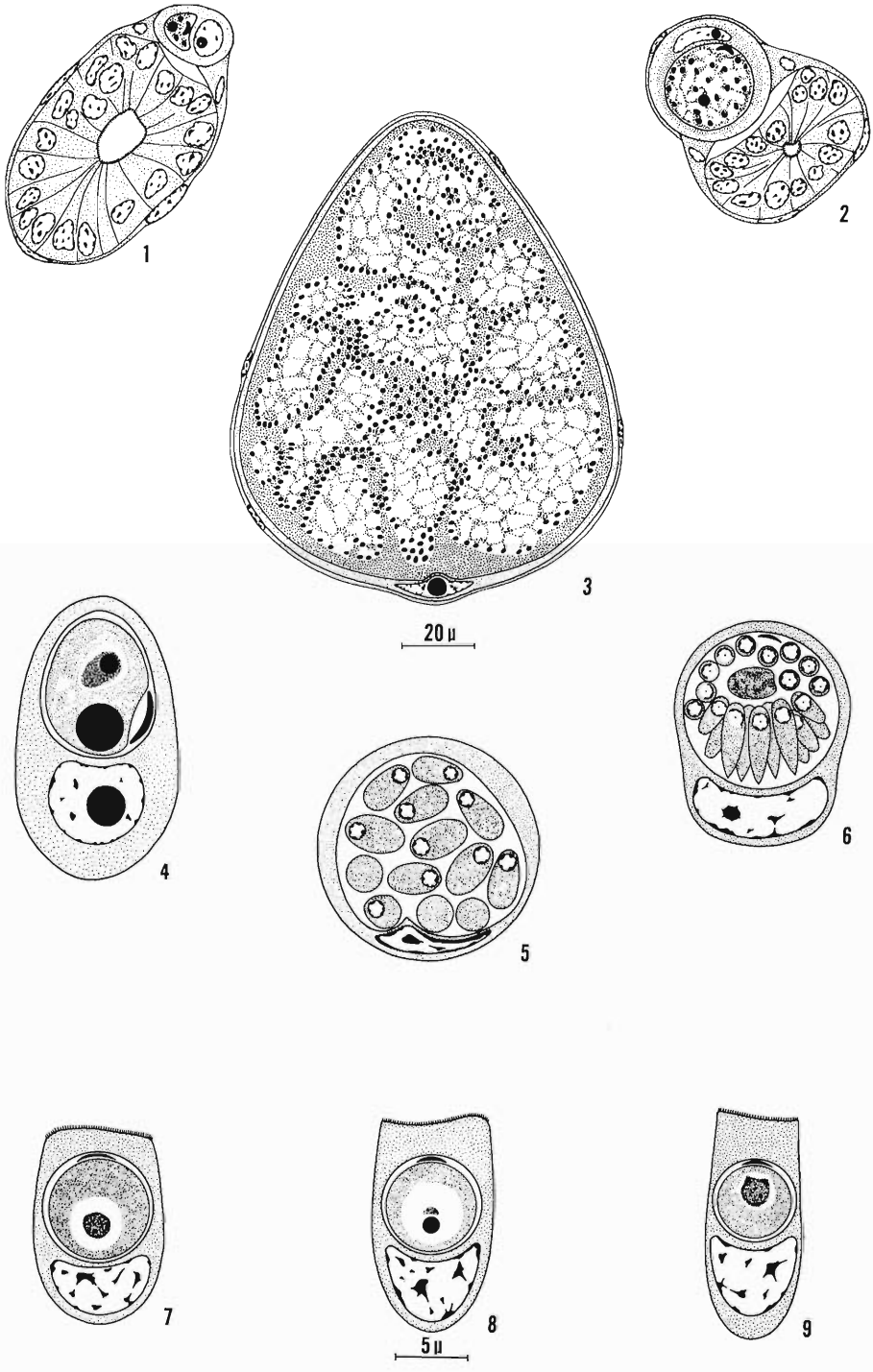
Figures 1–9. Drawings of endogenous stages of *E. ninakohlyakimovae* from lamb intestinal sections fixed with Zenker's fluid and stained with hematoxylin and eosin, unless otherwise stated. Figures 1–3, 20 micron scale; Figures 4–9, 5 micron scale. Duration of infection indicated in parentheses. 1. Early first-generation schizont with 3 nuclei; note relationship of host cell to reticular connective tissue cells surrounding crypt, refractile body in parasite, and crescent body in parasitophorous vacuole. Intestinal fistula (3 days). 2. First-generation schizont in host cell with cytoplasm arranged in 2 concentric layers; note flattened envelope around portion of host cell (5 days). 3. First-generation schizont; note altered host cell nucleus, envelope of flattened cells, and presence of parasitophorous vacuole containing eosinophilic, homogeneous material (heavy stippling) around schizont and between infolded layers of nuclei (7 days). 4. First-generation trophozoite in host cell with large nucleolus; note refractile body (below) and nucleus (above) in parasite and crescent body in parasitophorous vacuole. Fistula (3 days). 5. Macrophage from an invaded schizont, with rounded first-generation merozoites inside vacuole (12 days). 6. Mature second-generation schizont in epithelial host cell; note crescent body, residual body, and crescent-shaped pattern of chromatin in some merozoites (11 days). 7. Early trophozoite of second-generation schizont in epithelial host cell; note crescent body in parasitophorous vacuole. Cecal biopsy (24 hr). 8. Early macrogamete in epithelial host cell, with a satellite body located adjacent to parasite nucleolus; note crescent body (12 days). 9. Early microgametocyte in epithelial host cell; note crescent body (13 days).



Zenker's fluid and stained with iron hematoxylin, H and E, or with the method of Feulgen were measured with the aid of an ocular micrometer.

Results

Duration of Experimental Infection

In each of 2 lambs, oocysts were found in the feces from 12 to 18 days after inoculation. Thus, the prepatent period was 11 days and the patent period 7 days. The peak number of oocysts discharged occurred at a mean of 13.5 (13 to 14) days after inoculation. Two other lambs in the study also had a prepatent period of 11 days. The former 2 lambs became reinfected when reinoculated 1 month later.

Development of First-Generation Schizonts

All measurements in the following descriptions are given in microns, with the ranges in parentheses; unless otherwise stated, fixed preparations were used. First-generation schizonts underwent development in the reticular connective tissue cells of the lamina propria in the small intestine. Parasites were seen only in those reticular cells which were a part of the supporting envelope of connective tissue cells immediately surrounding and adjacent to the base of the intestinal crypts (Figs. 1, 2, 10, 29). The host cell harboring the developing schizont characteristically indented the adjacent epithelial layer of the crypt so that it bulged into the crypt lumen. First-generation schizonts were most numerous in sections of the ileum 1.5 to 4.5 m anterior to the ileocecal valve; smaller numbers occurred in some 6- to 7.5-m sections. Living, mature schizonts appeared macroscopically as small bodies beneath the mucosal surface of the intestine.

Trophozoites and early schizonts of the first generation

The earliest observed endogenous stages were trophozoites in intestinal fistulas from the 2 lambs killed 2½ days and 3 days after inoculation (Figs. 4 and 27). The trophozoites were more numerous in the latter, and early schizonts were also present in this lamb. In tissues from lambs inoculated *per os* and killed 3 days after inoculation, the only stages seen were a few early schizonts. No coccidia were observed in lambs inoculated *per os* and killed earlier than this. Five trophozoites averaged 8.5 (7.5 to 9.0) by 6.0 (5.0 to 6.5). Each had a single refractile body and a nucleus with a nucleolus. A crescent body was present in the parasitophorous vacuole. In trophozoites stained with iron hematoxylin, the nucleoplasm stained more intensely and more homogeneously than did the cytoplasm, which appeared light gray. The nucleolus stained more intensely with iron hematoxylin and with H and E than did the

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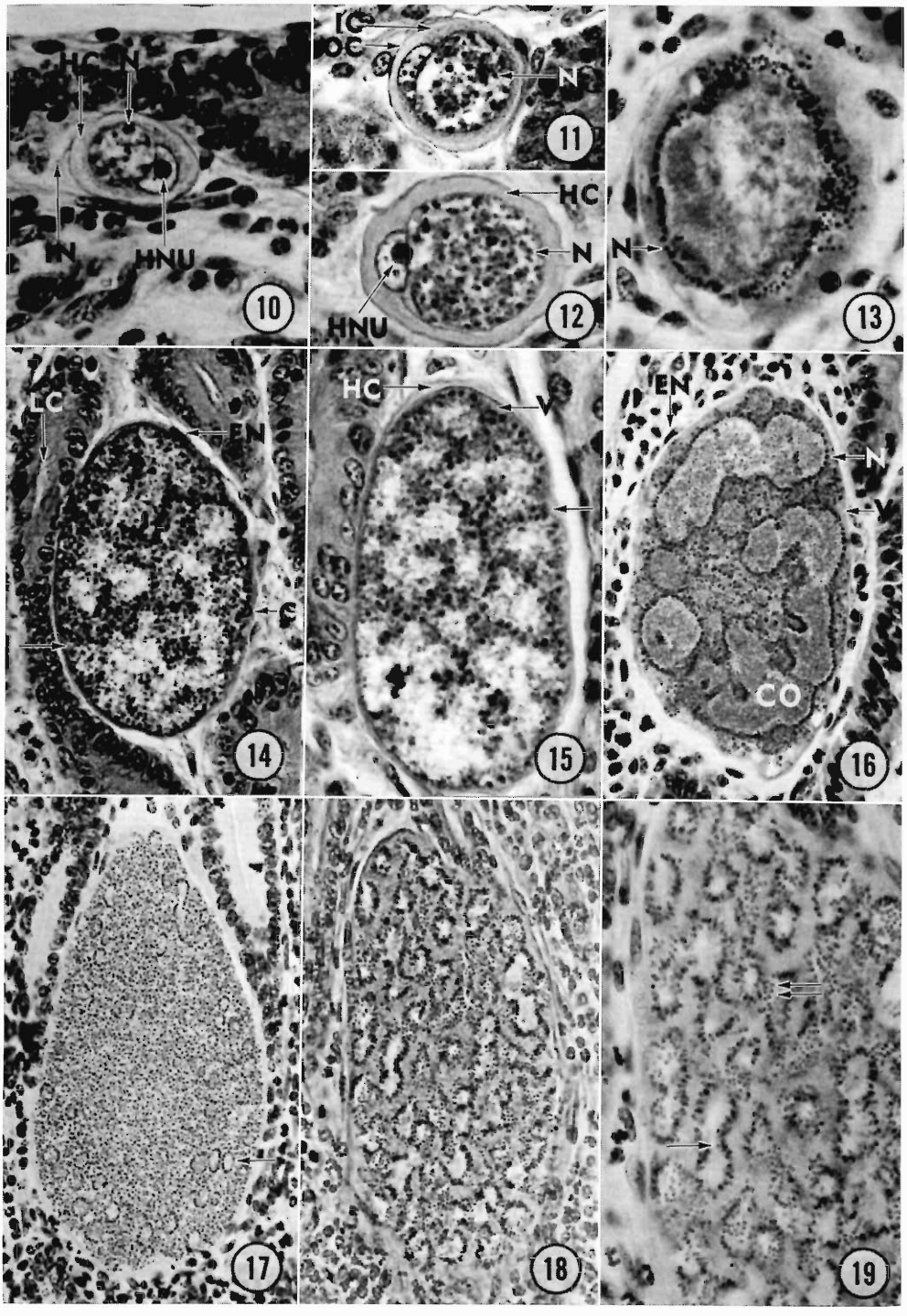
Figures 10–19. Photomicrographs of endogenous stages of *E. ninakohlyakimovae*, fixed with Zenker's fluid and stained with H and E unless otherwise noted. Intervals between inoculation and fixation of tissue indicated in parentheses. Abbreviations: C, crescent body; CO, compartment; CR, reticular connective tissue cell; EN, nucleus belonging to envelope of flattened cells; G, cytoplasmic granule; HC, host cell cytoplasm; HN, host cell nucleus; HNU, host cell nucleolus; IC, inner layer of host cell cytoplasm; IN, indentation of crypt wall; L, accumulation of leucocytic cells; LC, lumen of crypt; M, merozoite; MG, microgametes; N, nucleus of parasite; NL, nucleus of leucocytic cell; NU, nucleolus of parasite; OC, outer layer of host cell cytoplasm; R, refractile body; RB, residual body; SB, satellite body; V, parasitophorous vacuole. Figures 10–18. Schizonts. 10. Schizont, with random arrangement of nuclei; note characteristic indentation of crypt wall (5 days). Iron hematoxylin. × 600. 11. Schizont as in Fig. 10, but with stratification of host cell cytoplasm into two layers. × 600. 12. Schizont with random arrangement of nuclei and large nucleolus within hypertrophied host cell nucleus (6 days). × 600. 13. Schizont with nuclei arranged in a peripheral layer (7 days). Iron hematoxylin. × 600. 14. Schizont in early stage of compartmentalization; note crescent body and infoldings of peripheral layer of nuclei (arrow). × 425. 15. Schizont as in Fig. 14; note infoldings of peripheral layer of nuclei (arrow) (7 days). × 600. 16. Schizont in more advanced stage of compartmentalization. × 400. 17. Schizont in stage of blastophore formation; small rings of nuclei represent blastophores (arrow) (8 days). × 400. 18. Schizont in early stage of merozoite formation. Feulgen, phase contrast. × 400. 19. Enlarged portion of schizont shown in Fig. 18; note rows of nuclei in longitudinal sections of blastophores (arrow) and groups of nuclei in tangential sections of blastophores (double arrow). × 600.



nucleoplasm. With H and E, the cytoplasm appeared granular. Numerous Feulgen-positive granules were present around the nucleolus, which was Feulgen-negative. The refractile body and the crescent body stained intensely with iron hematoxylin, and eosinophilic with H and E, especially at the margin; they were Feulgen-negative.

The cytoplasm of the host cell was hypertrophied, and the nucleus enlarged (Fig. 4). The nucleolus of the host cell was markedly larger than normal. Most of the chromatin of the host cell was thinly distributed at the periphery, but some small chromatin granules were scattered within the nucleoplasm. In nuclei of similar non-infected cells, the peripheral layer of chromatin appeared thicker, and the granules of chromatin in the nucleoplasm larger.

In tissues from lambs killed 4 days after inoculation, numerous young schizonts with 3 to 9 nuclei were observed (Fig. 1). The schizonts in the 3-day fistula (Fig. 2) were at a similar stage of development. At this stage, the adjacent cells of the reticular connective tissue sheath of the crypt were attached directly to the host cell, apparently anchoring it in a relatively fixed position (Figs. 1, 29). In 5-day lambs, schizonts had numerous nuclei randomly distributed within the cytoplasm (Figs. 2, 10, 11). These nuclei were similar to those of earlier stages but appeared smaller and had a more deeply stained nucleoplasm, so that nucleoli were difficult to distinguish. The cytoplasm appeared more granular and heterogeneous than in previous stages, and in some specimens it appeared vacuolated (Fig. 2). The cytoplasm of host cells harboring this stage had 2 distinct layers, the inner being more granular and dense than the outer (Fig. 11). A layer of flattened cells had begun to form around the free surface of the host cell (Fig. 2); later, this layer completely surrounded the host cell in most specimens (Figs. 14, 16). Schizonts in lambs killed 6 days after inoculation were larger than in those a day younger, but still had a random distribution of nuclei (Fig. 12). The hypertrophied host cell nucleus showed little chromatin, but the nucleolus was greatly enlarged and Feulgen-negative.

Intermediate first-generation schizonts

In schizonts from lambs killed 7 days after inoculation, the contents of the parasitophorous vacuole stained deeply with eosin, and some schizonts had a single, peripheral layer of nuclei (Fig. 13). Infoldings of this layer into the interior of the schizont occurred (Figs. 3, 14, 15). Inpocketings of the parasitophorous vacuole were present between infolded adjacent layers of nuclei in the interior of the schizont, as indicated by the presence of the intensely eosinophilic, homogenous contents of the vacuole in these locations (Figs. 3, 14, 15). Thus, the individual compartments formed by the infoldings were separated by spaces continuous with the parasitophorous vacuole. Later, the infoldings formed compartments, which were small in some specimens and large in others (Fig. 16). Each individual compartment consisted of an internal mass of schizont cytoplasm, which appeared granular and vacuolated in some places, a peripheral layer of nuclei, and a limiting membrane. The host cell cytoplasm was thinly stretched around the schizont (Fig. 15) and the host cell nucleus was flattened. Refractile bodies were not observed in this or later stages, and crescent bodies were seen only rarely (Fig. 14).

In schizonts observed 8 days after inoculation, structures with a peripheral layer of nuclei were seen; such structures were termed blastophores in *E. bovis* (Hammond, Ernst, and Miner, 1966). In some specimens, these were relatively small and spheroidal (Fig. 17) and in others they were larger, ellipsoidal, and apparently interconnected. Each blastophore

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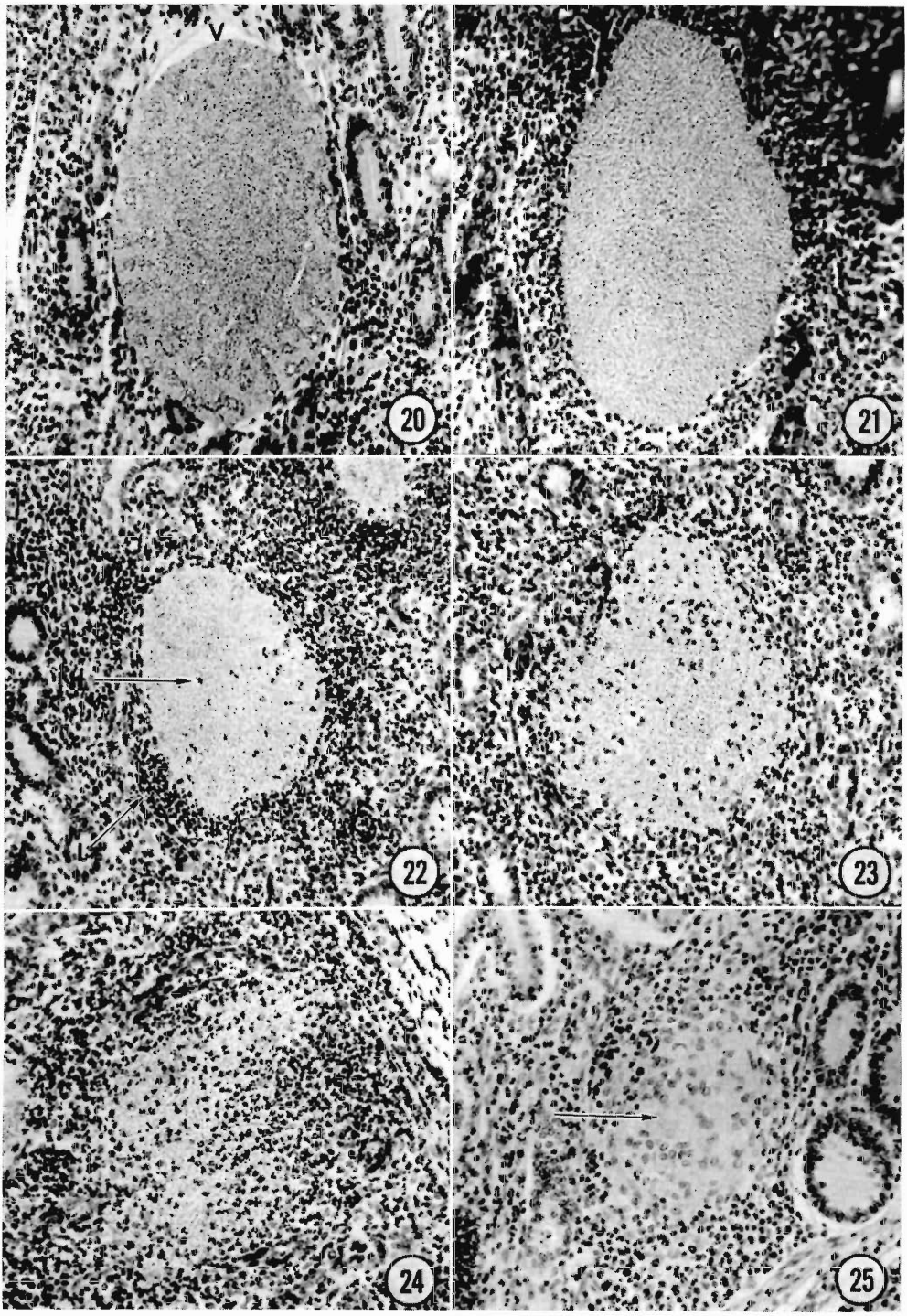
Figures 20–25. Schizonts. × 250. 20. Nearly mature schizont with patterned arrangement of merozoites at periphery and random arrangement in central area (9 days). Feulgen. 21. Mature schizont with merozoites randomly arranged throughout schizont; note moderate concentration of leucocytes surrounding the schizont (10 days). 21–23. Mature schizonts in early intermediate, and advanced stages, respectively, of invasion by leucocytic cells (10 days). Feulgen. 24. Site of destroyed schizont (arrow), with aggregation of macrophages and fibrocytic cells (13 days). Feulgen.



gave rise peripherally to merozoites. In schizonts in which merozoites were forming, the characteristic patterned arrangement of the nuclei was a prominent feature (Figs. 18, 19).

Mature first-generation schizonts

By 9 days after inoculation, formation of merozoites appeared to be complete, or nearly so. In some schizonts, the merozoites were arranged in ellipsoidal or spheroidal groups, probably indicating that they were still attached to residual bodies. In other schizonts, presumably more advanced, this arrangement was no longer present in the central portion (Fig. 20). The host cell cytoplasm appeared as a thin layer, about 1 to 2 μ in thickness. At 10 days after inoculation, schizonts had randomly distributed merozoites (Fig. 21). Fifty unfixed, mature schizonts were 290 (241 to 330) by 232 (188 to 285), and each had many thousands of merozoites.

Nearly all of the mature schizonts were surrounded by an accumulation of leucocytic cells (Fig. 21); this was first observed 10 days after inoculation. Various stages of invasion of the schizonts were seen at this time (Figs. 22, 23, 24). In lambs killed 10 to 14 days after inoculation, about 90% of the mature schizonts had some degree of invasion by eosinophils, neutrophils, and macrophages. The envelope of flattened cells and the host cell layer appeared to disintegrate as the leucocytic cells entered the schizont.

The merozoites within invaded schizonts were phagocytized by macrophages. Merozoites within macrophages had a more rounded shape than normal (Fig. 5). In some specimens, the cytoplasm of the merozoites was indistinct, and their nuclei could no longer be seen in more advanced stages. Thus, phagocytized merozoites were apparently destroyed by the macrophages. The sites of recently destroyed schizonts were indicated by aggregations of macrophages and fibrocytic connective tissue cells (Fig. 25). These aggregations were first observed 12 days after inoculation, indicating that destruction of schizonts may be completed in 2 days.

First-Generation Merozoites

One-hundred living, mature merozoites from 5 different schizonts measured 11.9 (10.5 to 12.5) by 2.1 (1.5 to 2.5). The nucleus was located in the posterior one-third of the merozoite (Figs. 26, 27). Numerous PAS-positive granules occurred in the middle one-third of the merozoite, and a few such granules were observed posterior to the nucleus. In control sections treated with diastase, these granules were PAS-negative. In acridine orange preparations, DNA-positive material was concentrated in 3 to 5 peripheral clumps in the nucleus. Flexing (Fig. 27) and gliding movements were observed in living merozoites, but not probing and pivoting movements.

Development of Second-Generation Schizonts

At 10 and 11 days after inoculation, second-generation schizonts were observed in epithelial cells lining the crypts in the cecum and colon (Figs. 30–34). Young schizonts were first seen 10 days after inoculation, and mature schizonts only on the 11th day. Development appeared to take 1 to 2 days. A few young and mature

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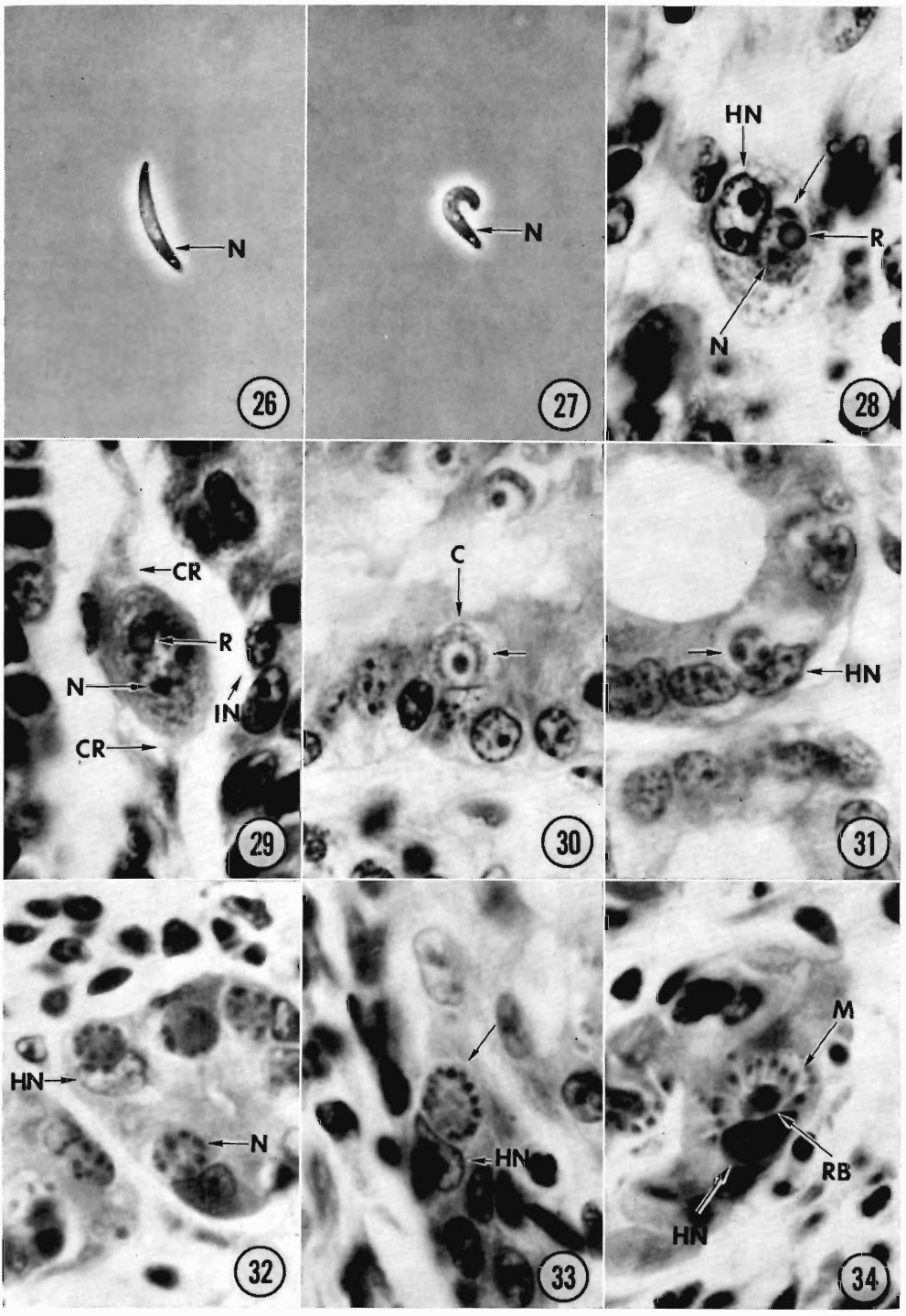
Figures 26–34. Asexual stages, fixed with Zenker's and stained with H and E unless otherwise noted. × 1,600. 26. Unfixed, first-generation merozoite in extended position (10 days). Phase-contrast. 27. Unfixed, first-generation merozoite in flexed position (10 days). Phase-contrast. 28. Trophozoite of first-generation schizont. Fistula (3 days). 29. Young, first-generation schizont; note attachment of adjacent reticular cells to host cell, and characteristic indentation of crypt wall. Fistula (3 days). 30. Trophozoite of second-generation schizont (arrow) in cecal biopsy tissue inoculated 24 hr earlier with first-generation merozoites. 31. Binucleate second-generation schizont (arrow) as in Fig. 29; note characteristic location of schizont in crypt epithelium. 32. Intermediate second-generation schizont with the majority of nuclei in a peripheral location (10 days). Iron hematoxylin. 33. Second-generation schizont in more advanced stage than that of Fig. 31, and with all nuclei in a peripheral location; note elevations at periphery (10 days). Iron hematoxylin. 34. Second-generation schizont with radially arranged immature merozoites still attached to eccentric residual body (10 days).



schizonts were observed in cells of the lamina propria.

Trophozoites and binucleate schizonts (Fig. 31), seen only in the 24-hr cecal biopsy sections, had nuclei which were basophilic with hematoxylin and eosin, with the most intense staining at the margins. No stages were observed in 36- or 48-hr cecal biopsies, or in the controls. The spheroidal trophozoites were 5.0 to 6.5 in greatest diameter and had a crescent body lying within the parasitophorous vacuole (Figs. 7, 30).

In intermediate schizonts, the nuclei were randomly distributed; later they were peripherally arranged (Fig. 32). Over each nucleus, an elevated area appeared at the surface of the schizont (Fig. 33). Each such area represented the site of a newly-forming merozoite, which grew radially into the parasitophorous vacuole, incorporating its respective nucleus (Fig. 34).

Mature schizonts had a crescent body, a compact central or eccentric residual body, and merozoites (Figs. 6, 34). Mature merozoites had small nucleoli. Feulgen-positive nuclear material was often arranged in the form of a crescent (Fig. 6). The host cell did not appear to be hypertrophied; its nucleus was characteristically indented in the area adjacent to the schizont. In sections, 30 schizonts (Fig. 6) were 12.0 (9.5 to 15.0) by 9.0 (6.5 to 12.0) and had an average of 24 (22 to 30) merozoites, 30 of which were 5.5 (5.0 to 6.5) by 1.4 (1.0 to 2.0).

Development of Microgametocytes

Microgametocytes were observed in the epithelial cells lining the crypts in the cecum and colon from 11 to 14 days after inoculation (Figs. 9, 35–40). A crescent body was present in the parasitophorous vacuole (Figs. 9 and 36). Nuclei of young microgametocytes stained intensely at the margins with hematoxylin, and several Feulgen-positive granules occurred in the nucleoplasm. The nuclei changed from a random distribution (Fig. 37) to a peripheral distribution (Fig. 38), and then elongated as microgamete formation began. A large residual body was present after completion of microgamete formation (Fig. 39). Mature microgametocytes, which were first seen 12 days after inoculation, had several hundred peripherally arranged microgametes (Fig. 40). Thirty mature microgametocytes were 15.0 (9.0 to 22.0) by 11.6 (8.0 to 15.0). The host cell appeared similar to that of second-generation schizonts.

Development of Macrogametes

Macrogametes occurred 11 to 14 days after inoculation in the same location as microgametocytes. In young specimens, a prominent nucleolus and associated satellite body occurred near the center of the large nucleus, which had lightly stained nucleoplasm (Fig. 41). The nucleolus stained intensely with iron hematoxylin and was basophilic with H and E. The satellite body stained less intensely with iron hematoxylin than the nucleolus, and was eosinophilic with H and E. The cytoplasm was granular, and a crescent body occurred in the parasitophorous vacuole.

In mature macrogametes, which were first observed 12 days after inoculation, eosinophilic granules were observed in the interior areas of the cytoplasm and basophilic granules at the periphery. The host cells were similar to those harboring microgametocytes. Thirty mature macrogametes were 16.1 (13.0 to 18.0) by 12.3 (10.0 to 14.5). The basophilic granules coalesced to form the outer layer of the oocyst wall (Fig. 42). This stage had a nucleus with a nucleolus and relatively dark nucleoplasm, with a deeply staining margin. Thirty oocysts (Fig. 43) in which granules were no longer present in the cytoplasm were 17.6 (15.0 to 20.5) by 13.3 (11.5 to 15.0).

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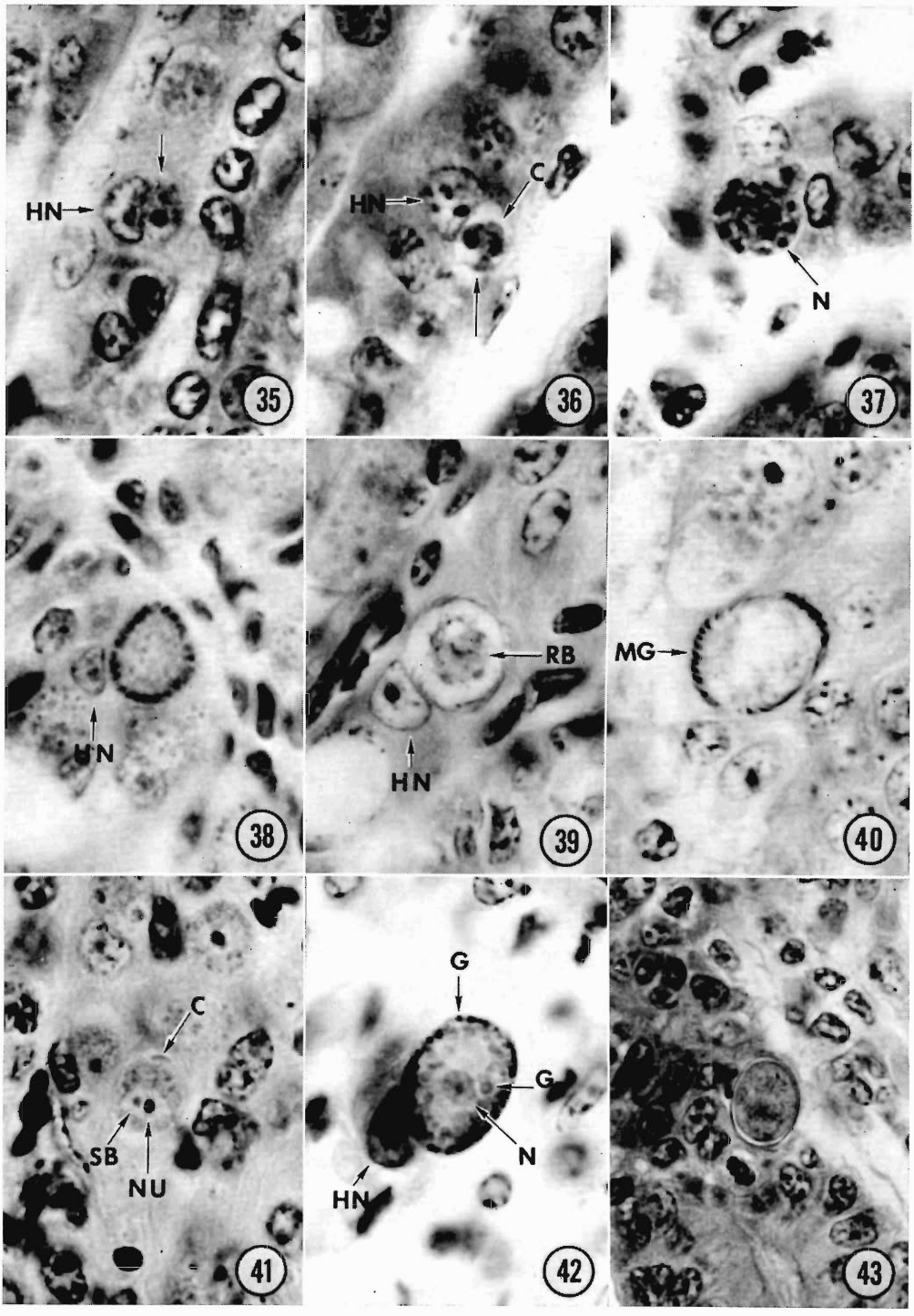
Figures 35–43. Sexual stages from colon of lambs harboring 13-day infections, in sections stained with H and E, × 1,600, unless otherwise noted. 35. Uninucleate microgametocyte (arrow). 36. Binucleate microgametocyte (arrow). 37. Immature microgametocyte with random arrangement of nuclei. 38. Immature microgametocyte with peripheral arrangement of nuclei. 39. Nearly mature microgametocyte with central residual body. Iron hematoxylin. 40. Mature microgametocyte; note microgametes. 41. Early macrogamete; note satellite body, nucleolus and crescent body (12 days). 42. Zygote with lightly stained eosinophilic granules and darkly stained peripheral basophilic granules. 43. Oocyst. × 1,000.



Discussion

The prepatent periods reported for *E. ninakohlyakimovae* in sheep by various authors differ considerably. Prepatent periods of 10 days (Christensen, 1941), 9 to 10 days (Hammond et al., 1967), 11 to 13 days (Svanbaev, 1967), 14 days (Krylov, 1961), and 15 days (Shumard, 1957) have been reported. Balozet (1932) reported a prepatent period of 10 to 13 days for this species in goats. In the present study a prepatent period of 11 days was observed. Oocysts were discharged for 7 days; similar results were obtained for 4 lambs by Svanbaev (1967). However, Hammond et al. (1967) reported that oocyst discharge continued in 15 lambs for 10 to 28 days. Also, Shumard (1957) reported that in 3 lambs harboring mixed infections with *E. ninakohlyakimovae* and *E. faurei*, oocyst discharge of both species increased until the twenty-first day, and then gradually decreased.

Lotze (1954) reported the occurrence of schizonts about 300 μ in diameter in the ileum of lambs experimentally infected with *E. ninakohlyakimovae*. He did not mention the location of these schizonts in the tissue, but stated that the sporozoites invaded cells apparently of endothelial nature at the base of the crypts. Singh and Pande (1967) reported endogenous stages of a species thought to be *E. ninakohlyakimovae* but their sheep had mixed infections.

The development of the large, first-generation schizonts of *E. ninakohlyakimovae* observed in sheep in the present study closely parallels that of the large first-generation schizont of *E. bovis* observed in cattle by Hammond et al. (1946) and Hammond et al. (1966). Mature first-generation schizonts of both species attained an average size of about 300 μ, had thousands of merozoites, and occurred in greatest concentration in the small intestine about 3 m anterior to the ileocecal valve. In both species a crescent body was present in the parasitophorous vacuole, and an envelope of flattened cells of host origin surrounded the host cells. However, the schizonts of *E. ninakohlyakimovae* developed more rapidly than those of *E. bovis*. The host cell harboring the first-generation schizont of *E. bovis* was identified by Hammond et al. (1946) as an endothelial cell lining the central lacteal within the villus, thus differing from that found for *E. ninakohlyakimovae* in the present study. However, the host cell reaction to the two species was similar, possibly because the host cells of each are mesodermal in origin. The reaction of the epithelial host cell of the first-generation schizont of *E. auburnensis* differs in that there is little or no cytoplasmic hypertrophy (Chobotar et al., 1969).

Hammond et al. (1966) reported that a layer of clear cytoplasm surrounded the peripheral layer of nuclei within the schizont of *E. bovis* before and during compartmentalization, and that this clear cytoplasmic layer became invaginated between the infolding layers of nuclei. In *E. ninakohlyakimovae*, a similar layer was observed, but was interpreted as the contents of the parasitophorous vacuole. This interpretation probably applies also to *E. bovis*. It is likely that the membrane which encloses the blastophore and later presumably forms the outer membrane of the merozoite is derived from the limiting membrane of the early schizont, as in *E. bovis* (Sheffield and Hammond, 1967). In this respect, the basic pattern of schizogony in *E. ninakohlyakimovae* and *E. bovis* would appear to be consistent with that observed in *Plasmodium* species by Hepler, Huff, and Sprinz (1966) and Vickerman and Cox (1967). The invasion of mature first-generation schizonts of *E. ninakohlyakimovae* by leucocytic cells was similar to that observed in *E. bovis* by Hammond et al. (1966), but occurred earlier, more frequently, and probably more rapidly in the former species. These differences might be associated with the deeper location of *E. ninakohlyakimovae* in the mucosa. In *E. bovis*, the schizonts located near the base of the mucosa were invaded more frequently than those in the villus.

Several species of *Eimeria* having large schizonts have been reported from sheep (Levine and Ivens, 1970). Lotze (1953) stated that at a certain stage of development of *E. ovina* (syn., *arloingi*), the nuclei of the large schizonts were arranged in rows, forming various configurations. Kotlan, Pellérdy, and Versenyi (1951) reported the formation of nests and spheres of nuclei in the large schizonts of *E. parva*. These findings indicate that in these 2 species, compartments or blastophores similar to those in *E. ninakohlyakimovae* are formed.

Chatton (1910) and Triffitt (1925) both stated that nuclei of *E. gilruthi* (syn., *Gastrocystis gilruthi*) schizonts became arranged in mulberry-form groups which then formed into spheres, called blastophores by Chatton. From these spheres, merozoites grew radially. These observations in *E. gilruthi* strongly suggest a pattern of schizogony similar to that reported for *E. ninakohlyakimovae* in the present study.

The development of second-generation schizonts of *E. ninakohlyakimovae* was similar to that of *E. bovis* as reported by Hammond et al. (1963). In both species, the schizonts develop in the epithelial cells of the crypts of the cecum and colon, and mature in 1 to 2 days. Crescent-shaped nuclei were not observed in *E. bovis*, but they were seen in the schizogonous stages of *E. nieschulzi* in rats by Matsubayasi (1938), and in *E. intestinalis* in rabbits by Cheissin (1958). The schizonts reported by Balozet (1932) and Sayin (1964) and the merozoites found by the latter in goats were larger than those of the second-generation we found in sheep. Therefore, it is likely that the species in goats is a different species from *E. ninakohlyakimovae* in sheep or, as stated by Levine and Ivens (1970), the two may be different strains or demes of the same species.

The location and development of the sexual stages of *E. ninakohlyakimovae* were similar to those of *E. bovis*, but these occurred earlier in the former species. Microgametogenesis in *E. ninakohlyakimovae* was similar to that observed in *E. bovis* (Hammond et al., 1946), and *E. caviae* (Lapage, 1940). The occurrence of a large, prominent nucleolus and satellite body in the nucleus of young macrogametes was reported for *E. bovis* (Hammond et al., 1946), *E. auburnensis* (Chobotar and Hammond, 1969), and *E. magna* (Cheissin, 1960). The significance of the satellite body has not been determined, although Cheissin (1960) found that it stained blue with bromphenol blue in *E. magna*. A crescent body as found in the present study in the parasitophorous vacuole of macrogametes and microgametocytes of *E. ninakohlyakimovae* also was observed in these stages in *E. auburnensis* (Chobotar and Hammond, 1969).

The numeroeus similarities between the endogenous stages of *E. ninakohlyakimovae* and *E. bovis* indicate the existence of a close phylogenetic relationship between these two species.

Acknowledgments

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In Memoriam

Harold Winfred Manter

June 18, 1898–April 15, 1971
Member since 1950

Some Hemiurid Trematodes of Marine Fishes from Ghana[1]

JACOB H. FISCHTHAL AND J. D. THOMAS[2]

ABSTRACT: Four new species in the digenetic trematode family Hemiuridae are described from marine fishes from Ghana: *Lecithocladium mecoderum*, *L. unibulbolabrum*, *Lecithaster africanus*, *L. ghanensis*. Nine previously described species reported are: *Parahemiurus merus*, *Dinurus barbatus*, *D. breviductus*, *D. tornatus*, *Ectenurus lepidus*, *E. virgulus*, *Lecithocladium augustiovum*, *L. excisum*, *Aponurus lagunculus*. New synonymy declared is *Parectenurus chloroscombri* and *Ectenurus trachuri* with *Ectenurus lepidus*, and *Aponurus trachinoti* with *A. lagunculus*. All previously described species represent new geographical distribution records; many new hosts are recorded.

The trematodes from Tema were fixed in corrosive acetate or Bouin's under coverslip pressure, stained in Ehrlich's acid hematoxylin or Mayer's carmalum, and mounted in balsam. All others were killed in hot water, transferred immediately to Lavdowsky's FAA fixative for 24 hr, and then stored in 70% alcohol plus 3% glycerine; whole mounts were stained in Mayer's carmalum and mounted in permount. An asterisk (*) preceding the host name indicates a new host record. All previously described species represent new geographical distribution records. Specimens have been deposited in the United States National Museum Helminthological Collection as noted. All measurements are in microns.

Parahemiurus merus (Linton, 1910) Woolcock, 1935

SYNONYMS: *Hemiurus merus* Linton, 1910; *Parahemiurus parahemiurus* Vaz and Pereira, 1930; *Parahemiurus platichthyi* Lloyd, 1938; *Parahemiurus atherinae* Yamaguti, 1938; *Parahemiurus harengulae* Yamaguti, 1938; *Parahemiurus noblei* King, 1962.

HOSTS: **Sardinella cameronensis* Regan, Cameroon sardine, **Ethmalosa dorsalis* (Cuvier and Valenciennes), shad (Clupeidae); **Engraulis encrasicholus* (L.), anchovy (Engraulidae); *Caranx hippos* (L.), jack or horse mackerel, *Selar crumenophthalmus* (Bloch), goggle-eye scad, **Trachinotus glaucus* (L.), palometa, **T. goreensis* Cuvier and Valenciennes, pampano (Carangidae); **Cynoglossus goreensis Steindachner*, tongue sole (Cynoglossidae); **Lagocephalus laevigatus* (L.), smooth puffer or globe-fish (Tetraodontidae); **Psettodes belcheri* Bennett (Psettodidae).

HABITAT: Stomach.

LOCALITIES: Cape Coast, Iture, Tema; Ghana.

SPECIMENS DEPOSITED: USNM Helm. Coll. Nos. 71674–71683 (one or more specimens from each host).

DISCUSSION: The most heavily infected host was *Sardinella cameronensis*. *Parahemiurus merus* has been reported from a variety of marine fishes, particularly clupeoids and carangids, from Southwest Africa, U. S. Atlantic, Gulf of Mexico, Bimini, Puerto Rico, Jamaica, Curaçao, Brazil, Ecuador, U. S. Pacific, Japan, and S. China, Okhotsk, and Bering Seas. Overstreet (1969) reported progenetic metacercariae of *P. merus* in the coelom of the chaetognath *Sagitta hispida* Conant from Biscayne Bay, Florida. As Sogandares and Hutton (1959) noted, this trematode is perhaps originally a parasite of the Clupeoidei. No doubt many records of *P. merus* are from fishes temporarily infected by feeding on clupeoid fishes harboring the adult worm as Manter (1954) suggested, or by ingesting progenetic metacercariae.

Dinurus barbatus (Cohn, 1903) Looss, 1907

SYNONYM: *Lecithocladium barbatum* Cohn, 1903.

HOST: *Coryphaena hippurus* L., dolphin (Coryphaenidae).

HABITAT: Stomach.

LOCALITY: Tema, Ghana.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 71684.

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DISCUSSION: This species has been reported from *C. hippurus*, *C. equisetis* L., *Sarda* (= *Pelamys*) *sarda* (Bloch) (Thunnidae), and *Paralabrax maculatofasciatus* (Steindachner) (Serranidae) from the European Atlantic, Gulf of Mexico, Puerto Rico, Cuba, Curaçao, and Mexican and Panama Pacific.

Dinurus breviductus Looss, 1907

HOST: *Coryphaena hippurus*.
HABITAT: Stomach.
LOCALITY: Tema, Ghana.
SPECIMENS DEPOSITED: USNM Helm. Coll. No. 71685.
DISCUSSION: This form has been found in *C. hippurus*, *C. equisetis*, *Sarda sarda*, and *Clupea melanostoma* (Clupeidae) from the European and U. S. Atlantic, Gulf of Mexico, Puerto Rico, Cuba, Curaçao, Argentina, and Red Sea.

Dinurus tornatus (Rudolphi, 1819) Looss, 1907

SYNONYMS: *Distomum tornatum* Rud., 1819; *Lecithocladium tornatum* (Rud.) Lühe, 1901.
HOST: *Coryphaena hippurus*.
HABITAT: Stomach.
LOCALITY: Tema, Ghana.
SPECIMEN DEPOSITED: USNM Helm. Coll. No. 71686.
DISCUSSION: This species has been recovered from *C. hippurus*, *C. equisetis*, *Sarda sarda*, and *Peprilus paru* (L.) (Stromateidae) from the European and U. S. Atlantic, Azores, Gulf of Mexico, Bimini, Puerto Rico, Cuba, Curaçao, Red Sea, and Gulf of Aden. All three species of *Dinurus* Looss, 1907, listed herein were from the same individual dolphin.

Ectenurus lepidus Looss, 1907

SYNONYMS: *Parectenurus chloroscombri* Siddiqi and Cable, 1960; *Ectenurus trachuri* Nikolaeva and Kovaleva, 1966.
HOSTS: *Chloroscombrus chrysurus* (L.), bumper, **Decapterus rhonchus* (Geoffroy St. Hilaire), mackerel scad (Carangidae); **Galeoides decadactylus* (Bloch), threadfin (Polynemidae).
HABITAT: Stomach.
LOCALITIES: Cape Coast (*C. chrysurus*), Tema (others); Ghana.
SPECIMENS DEPOSITED: USNM Helm. Coll. Nos. 71687–71689.
DISCUSSION: This species has been reported from a variety of marine fishes (mostly carangids) from the Mediterranean, Adriatic, and Black Seas, Brazil, New Zealand, Hawaii, and Gulf of Aden. We declare *Parectenurus chloroscombri* Siddiqi and Cable, 1960, based on a single worm from *Chloroscombrus chrysurus* from Puerto Rico, and *Ectenurus trachuri* Nikolaeva and Kovaleva, 1966, from *Trachurus mediterraneus* (Steindachner) (Carangidae) from the Mediterranean, Tyrrhenian, and Adriatic Seas synonyms of *Ectenurus lepidus*. Siddiqi and Cable (1960) placed their new species in the genus *Parectenurus* Manter, 1947, but Manter and Pritchard (1960) declared it a synonym of *Ectenurus* Looss, 1907. The former authors separated their form on the basis of an undivided seminal vesicle, but stated in the description that it had "shallow constrictions but not divided into distinct divisions." In our material from the same host species the division of the seminal vesicle varied from that described by Siddiqi and Cable to a distinct tripartite structure. Comparison of our specimens with two of *Ectenurus lepidus* from *Decapterus pinnulatus* (Eydoux and Soulayet) reported by Manter and Pritchard (1960) from Hawaii (kindly loaned by Dr. Mary Hanson Pritchard, University of Nebraska) and with the single specimen of *Parectenurus chloroscombri* (USNM Helm. Coll. No. 39397) show them to be basically alike. Nikolaeva and Kovaleva (1966) noted that their new species is most closely related to *E. lepidus*, but differs in having a sucker ratio of 1:3–4, in the ovary being larger than the testes, in lacking padlike thickenings on the anterodorsal part of the body, and in the more posterior extension of the ceca into the ecsoma. In our material the ovary varies from much smaller than the testes to much larger; the ceca extend into the ecsoma variable distances; only a few specimens show anterodorsal padlike thickenings; and the sucker ratios are usually slightly less than 1:3.0. Manter and Pritchard (1960) noted that the padlike thickenings are inconspicuous and not always evident; additionally, they noted that the sucker ratio is 1:2.8–3.0 in Manter's (1954) specimens from New Zealand.

Ectenurus virgulus Linton, 1910

HOSTS: **Caranx africanus* Steindachner, African horse mackerel, *C. crysos* (Mitchill), jack or horse mackerel, **Trachinotus glaucus* (Carangidae).

HABITAT: Stomach.

LOCALITY: Cape Coast, Ghana.

SPECIMENS DEPOSITED: USNM Helm. Coll. Nos. 71690–71692.

DISCUSSION: The differences cited by Manter (1947) between this species and *Ectenurus lepidus* Looss, 1907, were noted in our material. The padlike thickenings on the anterodorsal part of the body were prominent on all but a few of our specimens. Comparison of our worms with some of the original specimens collected by Linton (1910) from *Clupanodon pseudohispanica* (Poey) (Dorosomidae) from Tortugas, Florida (USNM Helm. Coll. No. 8508) show them to be basically similar. *E. virgulus* has been reported from a variety of marine fishes from the U. S. Atlantic, Gulf of Mexico, Bahama, Bimini, Bermuda, Jamaica, Curaçao, and Argentina.

Lecithocladium augustiovum Yamaguti, 1953

HOSTS: **Upeneus prayensis* Cuvier and Valenciennes, red mullet or goatfish (Mullidae); **Trachinotus glaucus*, **T. goreensis*.

HABITATS: Stomach, small intestine.

LOCALITIES: Tema (all hosts), Elmina (*U. prayensis*); Ghana.

SPECIMENS DEPOSITED: USNM Helm. Coll. Nos. 71693–71695.

DISCUSSION: Our specimens keyed to *L. augustiovum* in the key to the species of *Lecithcladium* Lühe, 1901, given by Reid, Coil, and Kuntz (1966). This species has been reported from scombrid and carangid fishes from Celebes and the Philippine Islands.

Lecithocladium excisum (Rudolphi, 1819) Lühe, 1901

SYNONYMS: *Lecithocladium excisiforme* Cohn, 1903; *L. gulosum* (Linton, 1899) Looss, 1907; *L. cristatum* (Rudolphi, 1819) Looss, 1907; *L. crenatum* (Molin, 1859) Looss, 1907.

HOSTS: **Ilisha melanota* Derscheid, long-finned herring (Clupeidae); **Scomberomorus tritor* (Cuvier and Valenciennes), Spanish mackerel or kingfish; *Scomber colias* Gmelin, Spanish or chub mackerel (Scombridae); **Caesiomorus* (= *Lichia*) *glaucus* (L.), leerfish (Carangidae); **Galeoides decadactylus*.

HABITATS: Stomach, small intestine.

LOCALITY: Tema, Ghana.

SPECIMENS DEPOSITED: USNM Helm. Coll. Nos. 71696–71700.

DISCUSSION: This species has been reported from a wide variety of marine fishes from the Baltic, North, Irish, Mediterranean, Adriatic, and Black Seas, New Zealand, Vietnam, Japan, South-West Africa, European and U. S. Atlantic, and Gulf of Mexico.

Lecithocladium mecoderum sp. n. (Fig. 1)

HOST: *Galeoides decadactylus* (Bloch), threadfin (Polynemidae).

HABITAT: Stomach.

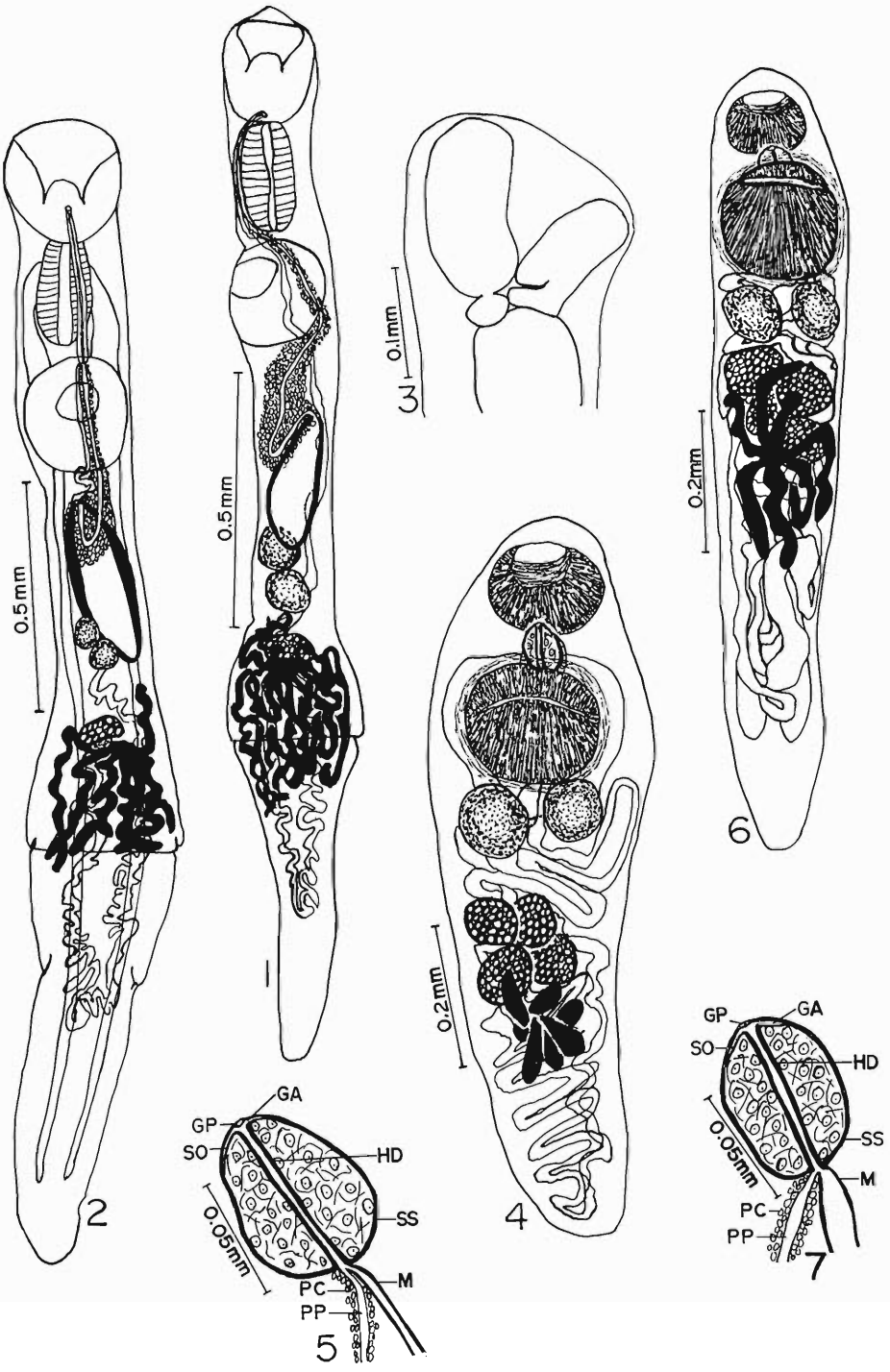
LOCALITY: Tema, Ghana.

DATE: 18 April 1964.

SPECIMEN DEPOSITED: USNM Helm. Coll. No. 71701 (holotype).

DIAGNOSIS (based on single adult specimen): Body without tegumental plications, elongate, very narrow almost to posterior part of body proper, latter part and anterior part of ecsoma swollen, remainder of ecsoma tapering to blunt point, preoral body pointed; total length 2,028, body proper 1,368 long, forebody 430 long, hindbody proper (without ecsoma) 755 by 175 at necklike postacetabular part and 265 at swollen posterior part, necklike postacetabular part 525 long, ecsoma 660 by 125 at midlength. Oral sucker subterminal ventral, with pair of distinct, deep, submedian incisions on ventral border forming lip, 193 by 180; acetabulum diameter 183; sucker length ratio 1:0.95, width ratio 1:1.02. Prepharynx absent; pharynx elongate, cylindrical, extending almost to acetabulum, 190 by 120; esophagus very short; ceca extending to near posterior extremity of ecsoma.

Testes two, smooth, contiguous, diagonal, nearly round, in necklike postacetabular part of hindbody just anterior to swollen part; anterior testis 80 by 85, lying 350 postacetabular; posterior testis 92 by 86. Seminal vesicle 280 by 90, saccular, walls 4–12 thick, very muscular, filling length of middle half of necklike postacetabular part of hindbody, overlapping anterior testis ventrally, lying 135 postacetabular. Pars prostatica very long, sinuous, proxi-



mal part with posterior loop along dextral side of seminal vesicle; prostate cells large, abundant around pars prostatica from origin to level of posterior third of acetabulum, cells smaller and fewer remainder of length. Hermaphroditic duct long, slender, commencing at level of posterior part of pharynx, following dextral side of latter, enclosed in thin walled sinus sac. Genital atrium shallow. Genital pore median, at posterior part of oral sucker.

Ovary smooth, transversely oval, 70 by 115, lying 42 posttesticular in anterior swollen part of hindbody proper. Seminal receptacle oval, anterodorsal to ovary, 85 by 60. Vitellaria with seven long, tubular, winding lobes, four right, three left, mainly postovarian, extending anteriorly to space between posterior testis and ovary and posteriorly into swollen part of ecsoma. Uterus extending posteriorly just beyond midlength of ecsoma, coiling mostly confined to ecsoma but with few coils in ascending limb lying between ovary and posterior testis as it passes from right side of ovary to left side of posterior testis, ascending as slightly sinuous duct sinistral to testes, seminal vesicle, pars prostatica and acetabulum, uniting with pars prostatica within sinus sac near its posterior end. Eggs yellow-brown, 12 measuring 15–18 by 7–10.

DISCUSSION: Although tegumental plications are lacking we are assigning this species to *Lecithocladium* Lühe, 1901, as all other characteristics of the genus are present. Reid, Coil, and Kuntz (1966) noted for their new species *L. bulbolabrum* that the presence, absence, or extent of the plications was very variable, and that caution should be exercised in using this characteristic for species of this genus. They further indicated that studies may possibly eliminate plications as a generic characteristic. Our species differs from all others in the genus in body shape. In the key to 18 species of *Lecithocladium* given by Reid, Coil, and Kuntz, assuming that the oral sucker is the same size as the acetabulum, our form came closest to *L. glandulum* Chauhan, 1945, from lutjanid and mugilid fishes from India. The latter differs further in having the oral sucker smaller than the acetabulum, the pharynx and cecal bifurcation considerably preacetabular, and longer eggs (24 by 10), in the sinus sac extending postpharyngeally and postbifurcally, and in the seminal vesicle occupying a much smaller part of the hindbody; the illustration of *L. glandulum* does show the posterior part of the hindbody proper slightly swollen. Assuming that the oral sucker is distinctly larger than the acetabulum, then our form keyed to a choice between *L. parviovum* Yamaguti, 1953, and *L. scombri* Yamaguti, 1953, from scombrid fishes from Celebes and Fiji. In regard to the key characteristic for body length it fits more closely the latter species, but for egg sizes given it overlaps both. Both species differ from ours in lacking any preoral body, and in the seminal vesicle occupying a much smaller part of the hindbody. *L. parviovum* differs further in the ecsoma being slightly longer than the body proper, and the sinus sac commencing dorsal to the acetabulum. *L. scombri* differs further in the ecsoma being only slightly shorter than the body proper. Our form superficially resembles the hemiurid genus *Mecoderus* Manter, 1940, in body shape, hence the species name (*meco*, long and *derum*, neck).

Lecithocladium unibulbolabrum sp. n. (Figs. 2, 3)

HOST: *Cephalacanthus volitans* (L.), flying gurnard (Dactylopteridae).

HABITAT: Stomach.

LOCALITY: Tema, Ghana.

DATES: 1, 6, 8 April 1965.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 71702 (holotype); No. 71703 (paratypes).

DIAGNOSIS (based on 29 adult specimens; six in ventral and three in lateral view measured so that measurements are length by width by depth): Body elongate, with ecsoma,

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Figures 1–7. ***Lecithocladium mecoderum*** **sp. n. Fig. 1. Whole mount, holotype, ventral view.** ***Lecithocladium unibulbolabrum*** **sp. n. Fig. 2. Whole mount, holotype, ventral view. Fig. 3. Anterior end of body showing oral sucker and part of pharynx, paratype, dextrolateral view.** ***Lecithaster africanus*** **sp. n. Fig. 4. Whole mount, holotype, ventral view. Fig. 5. Terminal genitalia, holotype.** ***Lecithaster ghanensis*** **sp. n. Fig. 6. Whole mount, holotype, ventral view. Fig. 7. Terminal genitalia, holotype.**

widest at vitellarian level; total length 1,985–3,435; body proper (without ecsoma) 1,320–2,085 by 350–565 by 385–510; ecsoma 665–1,650 by 230–470 by 235–520, length representing 28–48 per cent of total body length; forebody 380–635 long, hindbody (without ecsoma) 757–1,340 long, forebody–hindbody length ratio 1:1.3–2.9. Prominent glandular padlike thickening dorsal to oral sucker, projecting above body surface. Tegumental plications in only 14 worms on body proper but not ecsoma, on all of body proper in seven, postacetabularly only in seven but may be limited to short area. Oral sucker usually slightly longer than wide, 190–325 by 250–320 by 190–290, with pair of distinct submedian incisions on ventral border forming lip, dorsal part of sucker longer than ventral; when seen in lateral view dorsal lip only showing bulbous swelling posteriorly next to pharynx, bulb 27–36 long by 32–37 deep, not visible in dorsal or ventral mounts; ventral lip with dorsal groove short distance anterior to posterior margin but no ventral groove when observed in lateral view, grooves sometimes visible in dorsal or ventral mounts. Acetabulum usually slightly wider than long and usually longer than deep, in ventral view with circular muscle band inside anterior and lateral borders but not posteriorly, in lateral view with circular muscle band inside anterodorsal, dorsal, and posterior borders but not anteriorly or ventrally, 175–265 by 230–285 by 150–255. Sucker length ratio 1:0.75–0.91, width ratio 1:0.80–1.04, depth ratio 1:0.79–0.96. Prepharynx absent; pharynx cylindrical, sometimes slightly widened anteriorly, contiguous with posterior margin of oral sucker, 125–255 by 110–145 by 85–130; esophagus short, saclike, thick walled, muscular, anterodorsally directed, lying posterodorsal to pharynx; ceca emerging from anteriormost lateral parts of esophagus, descending almost to posterior end of ecsoma.

Testes two, smooth, diagonal, contiguous, usually overlapping one another; anterior testis dextral, 61–185 by 63–175 by 104–200, lying 167–415 postacetabular; posterior testis sinistral, 53–190 by 58–190 by 111–180. Seminal vesicle elongate, saccular, 210–455 by 145–215 by 95–220, lying 45–145 postacetabular in all but one contracted worm in which it overlaps latter 25; walls very thick, muscular, 30–53 thick in ventral view, 24–60 in lateral. Pars prostatica recurved on anterior part of seminal vesicle, straight to slightly sinuous in ascent, surrounded by prostate cells throughout length, cells numerous and large posteriorly but sparse and small anteriorly, uniting with metraterm dorsal to acetabulum. Hermaphroditic duct long, straight to slightly sinuous, without loop, protrusible, running length of sinus sac. Latter thick walled, muscular, 203–564 by 25–33 by 29–34; thickest part of wall 8–11. Genital pore median to slightly submedian, at midlength of ventral lip of oral sucker or more anteriorly but short of its anterior margin.

Ovary bean-shaped in ventral view with concavity posteromedian to submedian, transversely elongate, 80–170 by 97–190 by 102–190, lying 21–100 posttesticular in seven worms, in one contiguous with posterior testis and in another contracted specimen overlapping testis 15, lying 185–510 anterior to body proper–ecsoma junction. Vitellaria consisting of two main vitelline masses lying posteroventral to ovary with seven long, tubular, winding lobes (three right, four left) emerging from them, may enter ecsoma. Pattern of uterine coiling variable; descending on right or left into ecsoma, ascending on opposite side to postovarian or ovarian level, then crossing or not between ovary and posterior testis, even when not crossing some coils always invading space between ovary and posterior testis, postovarian ascending and descending coils may cross one another, passing dorsal to seminal vesicle, few coils between latter and acetabulum. Metraterm short, muscular. Eggs numerous, 30 measuring 15–21 by 8–12.

Discussion: In the key to the species of the genus given by Reid, Coil, and Kuntz (1966) our specimens keyed to a possible choice between *L. seriolellae* Manter, 1954, *L. megalaspis* Yamaguti, 1953, and *L. excisum* (Rudolphi, 1819) Lühe, 1901, depending on the combination of characteristics, but did not fit all those given in the key. In regard to step 1 of the key some of our specimens have the oral sucker about the same size as the acetabulum, in others the latter is distinctly larger. In *L. seriolellae* the uterus does not enter the ecsoma (step 6); *L. megalaspis* does not have an esophageal swelling (step 6) and the genital pore is at the posterior margin of the oral sucker (step 9). *L. excisum* differs in lacking the bulbous swelling at the posterior end of the

dorsal lip of the oral sucker. Our species is closest to *L. bulbolabrum* Reid, Coil, and Kuntz, 1966, from a scombrid fish from Formosa, the only species described with bulbous swellings of the oral sucker; however, the latter has swellings on both the dorsal and ventral lips. *L. bulbolabrum* lacks the glandular pad-like thickening dorsal to the oral sucker. Because our form has a single bulbous swelling on the oral sucker we have named it *L. unibulbolabrum*.

Aponurus lagunculus Looss, 1907

SYNONYM: *Aponurus trachinoti* Manter, 1940.

HOST: **Trachinotus glaucus* (L.), palometa (Carangidae).

HABITAT: Stomach.

LOCALITY: Cape Coast, Ghana.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 71704.

MEASUREMENTS AND SOME PERTINENT DATA (based on two adult specimens containing many eggs): Body 550–645 by 138–185; forebody 185–215 long, hindbody 250–303 long, forebody–hindbody length ratio 1:1.35–1.41; oral sucker 62–68 by 61–73, acetabulum diameter 115–127, sucker length ratio 1:1.85–1.87, width ratio 1:1.74–1.85; pharynx 35–36 by 37–41; gonads contiguous, testes diagonal, ovary in tandem with anterior testis; anterior (left) testis 66–67 by 70–76; posterior (right) testis 62–70 by 85–90; sinus sac 42–51 by 28–34; ovary 50–83 by 80–94; seminal receptacle 49–60 by 50–62, median to ovary in one specimen, anterodorsal in other; postvitellarian space 90–94 long; uterus extending anteriorly dorsal to oral sucker in one worm; eggs 27–33 by 13–18, some tapering almost to point at one end, others oval; excretory arms uniting dorsal to oral sucker.

DISCUSSION: Our specimens are smaller than any previously described for this species. It has been reported from a variety of marine fishes from the Mediterranean and adjacent seas, Black Sea, South-West Africa, Gulf of Mexico, Red Sea, S. China Sea, and Celebes. We declare *Aponurus trachinoti* Manter, 1940, from carangid and batrachoidid fishes from the Mexican and Californian Pacific a synonym of *A. lagunculus*. Manter (1940, 1947) noted the great similarity between these two species and remarked that they may be found to be the same. The differences cited are minor ones and subsequent descriptions of both species eliminate these differences.

Lecithaster africanus sp. n. (Figs. 4, 5)

HOST: *Galeoides decadactylus* (Bloch), threadfin (Polynemidae).

HABITAT: Small intestine.

LOCALITY: Cape Coast, Ghana.

DATE: 7 February 1966.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 71705 (holotype); No. 71706 (paratype).

DIAGNOSIS (based on three adult specimens from one of 21 fish examined): Body elongate, smooth, unspined, without ecsoma, extremities round but sometimes anterior end pointed, 810–965 long by 265–310 wide at acetabular level. Forebody 155–235 long, hindbody 475–610 long; forebody–hindbody length ratio 1:2.0–3.3. Oral sucker subterminal ventral, transversely elongate, aperture transversely oval, 112–125 by 133–158; preoral space 29–35 long; acetabulum transversely elongate, aperture a transverse slit, 145–177 by 180–210, surrounded by body fold in ventral view, probably protruding from body surface when mounted in lateral view; sucker length ratio 1:1.21–1.42, width ratio 1:1.27–1.35; acetabulum diameter–body length ratio 1:5.0–5.4. Prepharynx absent; pharynx 85–87 by 74–78, overlapping oral sucker dorsally, may overlap acetabulum dorsally; esophagus short, recurved dorsally, relatively thin walled at emergence from pharynx, remainder thick walled, muscular; cecal bifurcation dorsal to posterior part of pharynx; each cecum arising from esophagus as very short, thin walled tube sharply demarked from enlarged, conspicuously cell lined portion following, extending into postvitellarian space.

Testes two, smooth, symmetrical to subsymmetrical, at posterior margin of acetabulum, lying ventral to ceca so that median part of each testis may be intercecal and lateral part extracecal; right testis 87–97 by 94–104; left testis 85–97 by 95–108. Seminal vesicle mainly posterior to acetabulum, overlapping latter slightly, transversely to longitudinally elongate, 48–75 by 63–82. Pars prostatica long, slightly sinuous, surrounded by prostate cells through-

out length. Hermaphroditic duct tubular, straight, thick walled, muscular, protrusible, within sinus organ, extending length of sinus sac. Latter pyriform, thick walled, muscular, 68–73 by 57–71, lying ventral or ventrolateral (left) to pharynx, entirely anterior to or slightly overlapping acetabulum. Genital atrium very small. Genital pore median to submedian (right or left) to posterior part of oral sucker.

Ovary with four smooth lobes, posterior to and separated from testes, overlapping ceca ventrally, overall dimensions 127–157 by 143–169, lobes 53–87 by 44–92. Seminal receptacle small. Vitellarium with seven elongate lobes extending in all directions, lying posteroventral to ovary, overall dimensions 145–175 by 107–157, dimensions of lobes lying in flat plane 60–103 by 32–46, about same length or longer than ovarian lobes; postvitellarian space 109–200 long. Uterine coils extending from level of posterior part of acetabulum to posterior extremity. Eggs yellow-brown, 12 measuring 13–18 (average 14.5) by 9–11 (average 9.75).

DISCUSSION: Srivastava (1966) reviewed the genus, validating nine species; two other species are *L. testilobatum* Manter, 1969, and *L. leiostomi* Overstreet, 1970. In the key to the species given by Srivastava our form keyed to *L. salmonis* Yamaguti, 1934, from a variety of marine fishes from Japan and the U. S. Pacific. The latter differs in the acetabular aperture being oval and the sinus sac round to oval, in the genital pore being at the posterior part of the pharynx, in possessing a voluminous seminal receptacle, and in having larger eggs (21–24 by 13–16).

Lecithaster ghanensis sp. n. (Figs. 6, 7)

HOSTS: Type, *Hyporhamphus calabaricus* (Günther), half-beak (Hemirhamphidae); *Cypselurus lutkeni* (Jordan and Evermann), flying-fish (Exocoetidae); *Trachinotus glaucus* (L.), palometa (Carangidae); *Periophthalmus koelreuteri* (Pallas), mud-skipper (Gobiidae).

HABITATS: Stomach (*H. calabaricus*), small intestine (others).

LOCALITIES: Kakum River estuary (*P. koelreuteri*) and Gulf of Guinea (others) at Iture, Ghana.

DATES: 17, 21 February, 20 April 1966.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 71707 (holotype, from *H. calabaricus*); No. 71708 (paratypes, *H. calabaricus*); No. 71709 (paratypes, *C. lutkeni*); No. 71710 (paratypes, *T. glaucus*); No. 71711 (paratype, *P. koelreuteri*).

DIAGNOSIS (based on one and four specimens from two of 12 *H. calabaricus* examined, all measured; one and three from two of five *C. lutkeni*, one measured; one and three from two of 17 *T. glaucus*, one measured; two from one of nine *P. koelreuteri*, one measured): Body elongate, very narrow, smooth, unspined, without ecsoma, extremities round, 658–1,287 by 160–250 at acetabular level. Forebody 100–200 long; hindbody 430–967, tapering to blunt point; forebody–hindbody length ratio 1:3.2–6.7. Oral sucker subterminal ventral, transversely elongate, aperture transversely oval, 70–93 by 87–107; preoral space usually between 20–30 long but none in one and only 7 in another; acetabulum transversely elongate, aperture a transverse slit, 120–160 by 128–195, projecting 65–97 above ventral body in five specimens mounted in lateral view, surrounded by body fold in ventral view; sucker length ratio 1:1.50–2.13, width ratio 1:1.45–1.82; acetabulum diameter–body length ratio 1:5.2–7.8. Prepharynx absent; pharynx 53–78 by 46–63, overlapping oral sucker dorsally, may overlap acetabulum dorsally; esophagus short, relatively thin walled at emergence from pharynx, remainder thick walled, muscular, recurved dorsally; each cecum arising from esophagus as very short thin walled tube sharply demarked from enlarged, conspicuously cell lined portion following, extending into postvitellarian space to within 105–225 of posterior extremity.

Testes two, smooth, symmetrical to subsymmetrical, contiguous, often overlapping, at posterior margin of acetabulum, ventral to ceca; right testis 73–110 by 62–90; left testis 73–125 by 61–90. Seminal vesicle usually entirely posterior to acetabulum but may overlap latter, 32–128 by 34–80. Pars prostatica long, slightly sinuous, surrounded by prostate cells throughout length. Hermaphroditic duct tubular, straight, thick walled, muscular, protrusible, within sinus organ, extending length of sinus sac. Latter elongate oval, thick walled, muscular, usually lying ventral or ventrolateral to pharynx, usually overlapping oral sucker and acetabulum dorsally, 51–82 by 30–53. Genital atrium very small. Genital pore variable in

position in single population of worms, depending in part on state of expansion or contraction of body, median to submedian (right or left) from level of esophagus to posterior part of oral sucker.

Ovary with four smooth lobes, posterior to testes, may be contiguous with latter, overlapping ceca ventrally, overall dimensions 133–220 by 105–155, lobes 63–109 by 44–77. Seminal receptacle small. Vitellarium with seven elongate lobes extending in all directions, lying posteroventral to ovary, overall dimensions 167–300 by 100–183, dimensions of lobes lying in flat plane 81–155 by 27–56, longer than ovarian lobes; postvitellarian space 132–462 long. Uterine coils extending from level of middle or anterior part of acetabulum to within 39–237 of posterior extremity. Eggs yellow-brown, 40 measuring 13–17 (average 15.25) by 9–12 (average 10.25).

DISCUSSION: In the key to the species of *Lecithaster* Lühe, 1901, given by Srivastava (1966) some of our specimens keyed to *L. salmonis*, while some keyed to a choice between *L. confusus* Odhner, 1905, from a variety of marine fishes from the North, Adriatic, Tyrrhenian, Mediterranean, Black, White, and Barents Seas, Nile, U. S. and Canadian Atlantic, Gulf of Mexico, and U. S. Pacific, and *L. indicus* Srivastava, 1935, from a clupeid freshwater fish from India, but would not entirely fit all the characteristics of either of them as given in the key. *L. ghanensis* differs from all known species in the genus, including *L. africanus* sp. n. which it also resembles, in possessing a very slender, elongate body so that the acetabulum, testes, ovary, and vitellarium nearly fill the body width at their respective levels. *L. africanus* differs further in having a pyriform sinus sac; *L. salmonis* in having a large seminal receptacle and a short postvitellarian space; *L. confusus* in possessing ovarian lobes with irregular undulating margins and vitelline lobes smaller than those of the ovary; and *L. indicus* in having testes with irregular undulating margins and vitelline lobes almost equal in length to those of the ovary.

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Freshwater Larval Trematodes. XXVI. Life Cycle of *Guaicaipuria pseudoconcilia* (Nasir, Díaz, and Lemus de Guevara, 1969) comb. n., gen. n., subfam. n.

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ABSTRACT: *Cercaria pseudoconcilia* Nasir, Díaz, and Lemus de Guevara, 1969, encysts in the gills of freshwater fishes: *Rivulus harti* and *Lebistes reticulatus*. These cysts, when fed to a laboratory raised pigeon, developed into adult flukes of the family Cathaemasiidae, but certain characters such as the absence of esophageal diverticula, location of the ventral sucker nearer to the anterior extremity than to midbody, and separation of the right and left vitelline fields in the preacetabular region, necessitated the introduction of a new genus, *Guaicaipuria*, and a new subfamily, Guaicaipuriinae. Since the larva was known before its adult, the species becomes *Guaicaipuria pseudoconcilia* (Nasir et al., 1969). The natural definitive and second intermediate hosts are unknown.

Nasir, Díaz, and Lemus de Guevara (1969) described a gymnocephalic cercaria, *C. pseudoconcilia*, from the freshwater snail, *Pomacea glauca* (L.), but its life cycle remained undetermined. During this research, the cercaria has been connected, experimentally, to the adult of a new genus, *Guaicaipuria*, of a new subfamily, Guaicaipuriinae. The natural definitive and second intermediate hosts are unknown.

Materials and Methods

The second intermediate host, the freshwater fish *Rivulus harti* (Boulenger), was collected from a stream, "Quebrada de Yaguaracual," en route to Puerto la Cruz, which lacks snails, and whose piscine fauna has never been found to harbor any kind of metacercariae. Five of the fish were exposed for 24 hr, to an undetermined number of *Cercaria pseudoconcilia*, and the other five were left as controls. After 8 days, at room temperature (26 C), the gill arches and gill filaments of the experimental hosts were heavily infected with metacercariae while the controls proved negative. These metacercariae, along with gills, were fed to 4 two-week old, laboratory raised pigeons, whose feces were then examined daily. On the 10th day the feces of one of the pigeons contained trematode eggs and was killed. Fifteen egg-bearing adults were recovered from its cloaca, whereas the other three pigeons proved negative by autopsy. Another freshwater fish, *Lebistes reticulatus* (Peters), also served as the second intermediate host, but the infection rate was relatively low with a maximum of 7 metacercariae from the gills of a fish.

The parasites were washed several times in Locke's solution, then fixed in Gilson's fixative (70 C), and stained with acetocarmine. Figures were drawn with the aid of a camera lucida; measurements are in millimeters.

Results

Guaicaipuria pseudoconcilia (Nasir, Díaz, and Lemus de Guevara, 1969)

Metacercaria (Fig. 1)

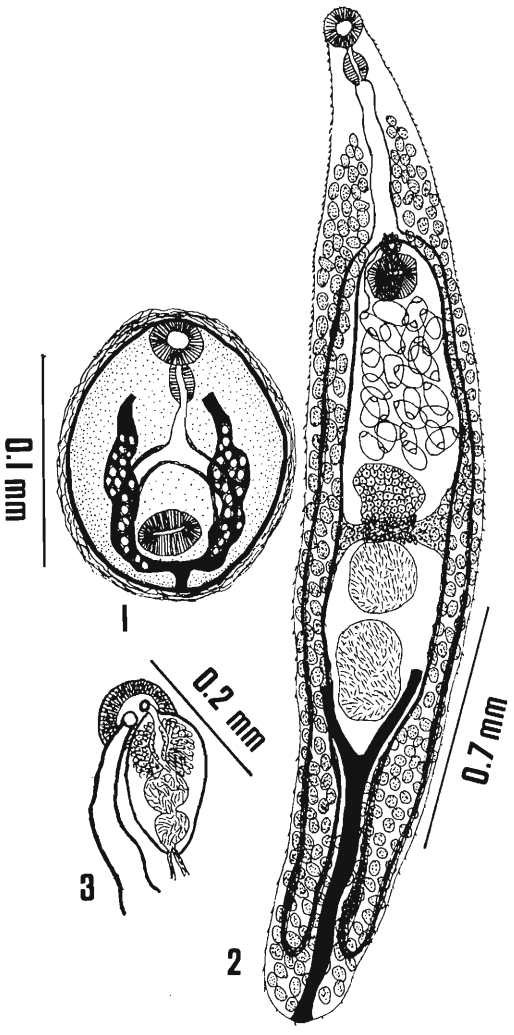
Cercariae of *G. pseudoconcilia* encysted in the gills of *Rivulus harti*. The cysts were oval, enclosed in a double-layered cyst wall; an internal delicate layer, the thickness of which remains constant, and an external fibrous layer of host origin, the thickness of which increases with time. The living cysts under slight cover glass pressure, excluding the cyst wall, are 0.132–0.138 by 0.120–0.117.

Adult (Figs. 2–3)

DEFINITIVE, EXPERIMENTAL HOST: Domestic pigeon, *Columba livia*.

NEOTYPE: USNM Helm. Coll. no. 70520.

[1] Supported in part by a grant, # DCC-69/69/DB-23, from Comisión de Desarrollo y Coordinación Científicas of Universidad de Oriente.



Figures 1–3. ***Guaicaipuria pseudoconcilia*****: Fig. 1. Metacercaria. Fig. 2. Adult. Fig. 3. Terminal genitalia, cirrus sac, and metraterm opening independently in a muscular genital pore.**

Description

Body spinose, with maximum width in region of ovary, attenuated anteriorly. Ventral sucker larger than oral, ratio 1:1.2, nearer to anterior extremity than to midbody. Prepharynx short. Pharynx smaller than oral sucker, with a ratio of 1:1.5. Esophagus without lateral diverticula. Ceca extending to posterior end of body. Ovary unlobed, transversely elongated, pretesticular, mesial, distinctly preequatorial, far posterior to base of cirrus pouch. Uterus intercecal, preovarian, opening independently into genital atrium (Fig. 3). Uterine eggs with fully developed miracidia. Anterior limits of vitellaria fluctuating between pharynx and halfway along esophagus, without confluence in preacetabular region, meeting slightly along median line posterior to testes. Testes elongated, rarely anterior testis somewhat spherical, unlobed, tandem, postequatorial. Cirrus sac lying anterior and dorsal to ventral sucker, may extend posterior to the latter, containing bipartite seminal vesicle and globular pars prostatica. Genital pore median, halfway between esophageal bifurcation and ventral sucker. Excretory vesicle Y-shaped, bifurcating short distance posttesticularly. Measurements of six egg discharging adults: body 2.560–3.200 by 0.384–0.544; oral sucker 0.084–0.103 in diam.; pharynx 0.056–0.065 in diam.; ventral sucker 0.094–0.141 in diam.; ovary 0.147–0.168 by 0.180–0.198; intrauterine eggs 0.093–0.123 by 0.051–0.066; anterior testis 0.150–0.263 by 0.150–0.206; posterior testis 0.178–0.272 by 0.141–0.188; cirrus sac 0.195–0.225 by 0.099–0.135.

Discussion

The cathaemasiids for which the life cycles are known are: (1) *Cathaemasia hians* (Rudolphi, 1809) Looss, 1899, (Szidat, 1939); (2) *Ribeiroia ondatrae* (Price, 1931) Price, 1942, (Kuntz, 1951); (3) *R. thomasi* (McMullen, 1938) Yamaguti, 1958, (syn. *Psilostomum ondatrae* Price, 1931, of Beaver, 1939); and (4) *R. marini* (Faust and Hoffman, 1934) Basch and Sturrock, 1969, (Basch and Sturrock, 1969). The cercaria of *Guaicaipuria pseudoconcilia* is readily distinguished from that of these four forms in the flame cell system, number of apertures of penetration ducts, and by the absence of lateral esophageal diverticula, and, in comparison with *C. hians*, lack of collar spines. A detailed account of the cercaria of *G. pseudoconcilia* has already been published, thus a redescription is unnecessary.

The general features of the flukes, involved in this investigation, fit into the family Cathaemasiidae Fuhrmann, 1928, but the subfamilies therein Cathaemasiinae Dollfus, 1950, Ribeiroiinae Travassos, 1951, Liliatrematinae Gubanov, 1954, and Reeselliinae Mettrick,

1963, fail to embrace these parasites principally in the location of the ventral sucker nearer to the anterior end of the body than midbody, the larger size of ventral sucker in relation to the oral one, and the right and left vitelline fields not being confluent preacetabularly. These differences lead to the erection of a new genus, *Guaicaipuria*, and a new subfamily, Guaicaipuriinae. The larval form of the species was named before the adult, thus the flukes stand as *Guaicaipuria pseudoconcilia* (Nasir et al., 1969).

According to Yamaguti (1958) the family Cathaemasiidae comprises three subfamilies, Liliatrematinae, characterized by a pentagonal hoodlike expansion of the oral sucker, and Ribeiroiinae and Cathaemasiinae which lack this structure. In Ribeiroiinae the esophagus bears a pair of lateral diverticula and the vitallaria occupy the pre- and postacetabular regions of the body while in Cathaemasiinae there are no such diverticula and the vitellaria are limited only to the postacetabular region. In the new genus, reported herein, the esophageal diverticula are lacking, but the vitelline glands have a distribution similar to that of Ribeiroiinae.

Mettrick (1963) introduced the subfamily Reeselliinae, for *Reesella doviensis* Mettrick, 1956, a parasite of the oystercatcher, *Himantopus ostralegus* Laubmann, 1923, from Wales, in which the esophageal diverticula are absent, the ventral sucker is smaller than the oral and is nearer to midbody than to anterior end, the vitellaria extend from the pharyngeal region to the posterior extremity, the follicles of both sides meet medially in front of the ventral sucker as well as posterior to the testes, and the ovary lies near the base of the cirrus sac. In *G. pseudoconcilia* the esophageal diverticula are also absent, but the ventral sucker is larger than the oral and is nearer to the anterior extremity than midbody, and the vitellaria are set distinctly apart in the preacetabular zone; moreover, the ovary lies a considerable distance posterior to the cirrus sac. Thus, a new subfamily and a new genus are being established with the following characters:

Guaicapuriinae subfam. n.

Cathaemasiidae. Oral sucker without hoodlike expansion. Ventral sucker nearer to anterior extremity than to midbody. Esophagus without lateral diverticula. Vitellaria occupying most of space in regions anterior and posterior to ventral sucker, not confluent preacetabularly. Cirrus sac voluminous, may extend posterior to ventral sucker, enclosing bipartite seminal vesicle.

Guaicapuria gen. n.

Cathaemasiidae, Guaicaipuriinae. Body attenuated anteriorly, maximum width in region of ovary. Ventral sucker larger than oral. Intestinal ceca extending posterior to testes. Ovary unlobed, always at considerable distance posterior to cirrus sac. Testes unlobed, tandem, postequatorial.

Modified Key to the Subfamilies of Cathaemasiidae (after Yamaguti, 1958, and Mettrick, 1963)

1. Oral sucker funnel shaped, with pentagonal hoodlike expansion ..... Liliatrematinae
   Oral sucker without hoodlike expansion 2
2. Esophagus with a pair of lateral diverticula, vitellaria in fore- and hindbody ..... Ribeiroiinae
   Esophagus without lateral diverticula ... 3
3. Vitellaria confined to hindbody ..... Cathaemasiinae
   Vitellaria very extensive in fore- and hindbody 4
4. Vitellaria confluent in midline in preacetabular region, acetabulum nearer to midbody than to anterior extremity ..... Reeselliinae
   Vitellaria not confluent in midline in preacetabular region, acetabulum nearer to anterior extremity than to midbody ..... Guaicaipuriinae

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Rogerus rosae sp. n. (Nematoda: Cylindrolaiminae) from Marathwada, India

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ABSTRACT: *Rogerus rosae* sp. n., collected from the soil around the roots of rose, differs from the other two known species, *R. orientalis* and *R. rajasthanensis*, in having a single tooth at the beginning of the stoma, in the absence of cephalic setae and in not possessing the glandular organs at the base of esophagus.

Hoeppli and Chu in 1932 proposed the genus *Greenia*, when they described the species *G. orientalis*. But, as the generic name *Greenia* was preoccupied in arthropods, Hoeppli and Chu in 1934 renamed the genus as *Rogerus*. Andrassy, 1959, for the same reason but unaware of this change, proposed the name *Greenenema* for *Greenia*. Goodey (1963) also retained the name *Greenenema*. Khera in 1966, while describing the new species *Rogerus rajasthanensis*, noted the change in name already made by Hoeppli and Chu in 1934. Thus the name *Rogerus* is accepted and a new species is described herein.

Rogerus rosae sp. n. (Fig. 1, A–F)

Measurements

Females (5): L = 0.473–0.507 mm; a = 24.8–28.8; b = 4.8–5.4; c = 3.4–3.6; V = 40.5–41.5.

Holotype female: L = 0.473 mm; a = 25.5; b = 4.9; c = 3.4; V = 41.

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Description

Female: Body slightly curved ventrally when relaxed, cylindrical, tapering towards both the extremities, more so posteriorly. Cuticle with fine transverse striations. Lateral fields absent.

Head continuous with body contour, rounded anteriorly; lips amalgamated. Circle of six papillae observed in *en face* view; cephalic setae absent. Amphids not discernible in lateral view, but pore-like openings seen in dorsoventral view, about 7 μ behind the anterior end. Stoma cylindrical, slightly narrowing posteriorly, about 30 μ long and armed with anteriorly directed dorsal tooth near mouth. Two slightly refractive thickenings situated at beginning of stoma, anterior to dorsal tooth. Cylindrical esophagus completely surrounding stoma and with pyriform basal bulb having sclerotized valvular apparatus. Esophago-intestinal valve rounded. Intestine with wide lumen. Rectum less than one anal-body-width long. Tail 9–10 anal-body-widths long, tapering gradually posteriorly to 'dagger-like' process at terminus, which is 11.5 μ long. Caudal glands present.

Vulva a transverse slit situated at 41% of

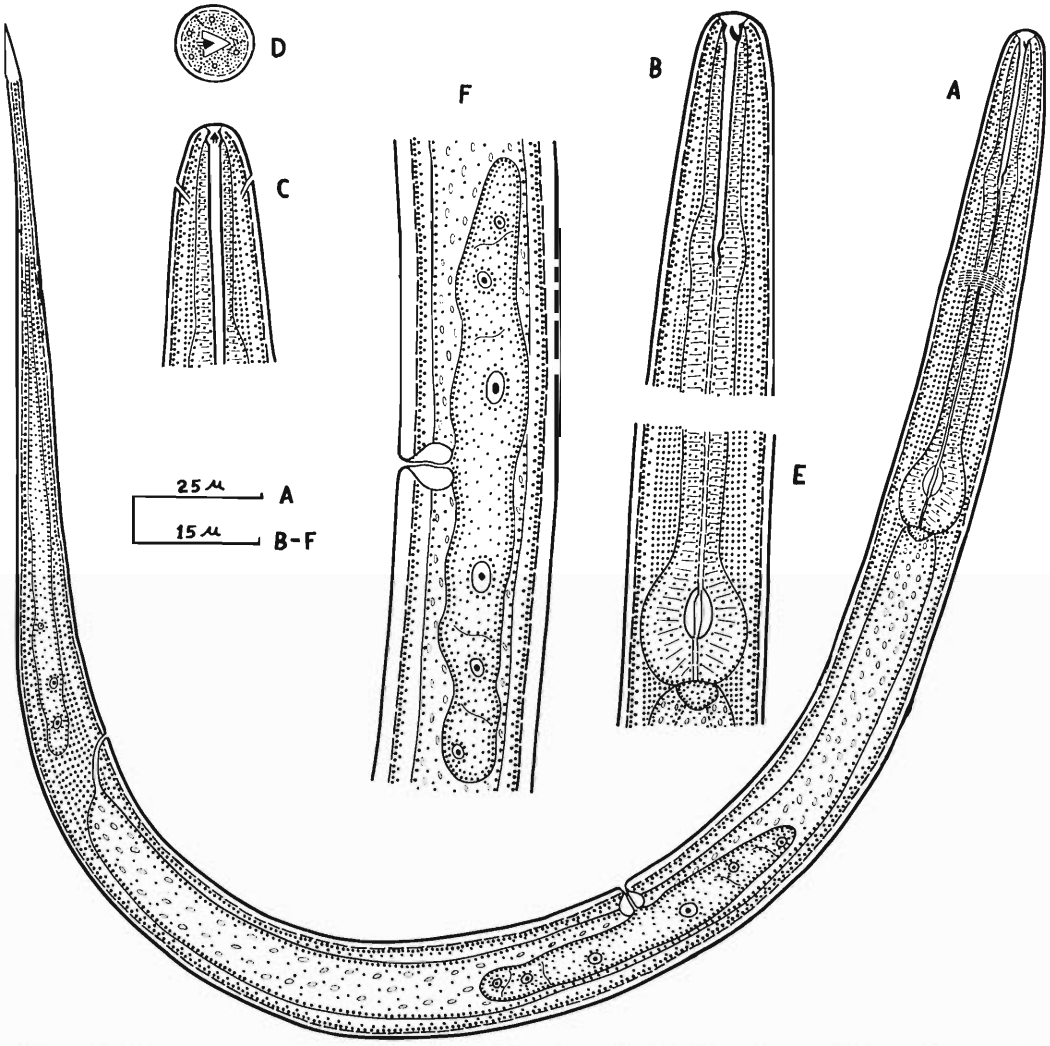

Figure 1. ***Rogerus rosae*** **sp. n. A. Female. B. Anterior extremity, lateral view. C. Anterior extremity, ventral view. D. En face view. E. Posterior esophageal region. F. Female gonadal region.**

body. Vagina at right angles to body axis, extending about one-third body width. Gonads amphidelphic and outstretched.

MALE: Not found.

TYPE HABITAT AND LOCALITY: Soil around roots of rose from Marathwada University Campus, Aurangabad, Maharashtra, India.

TYPE SPECIMENS: Holotype and five paratype females deposited in nematode collection of Zoology Department, Marathwada University, Aurangabad, Maharashtra, India.

RELATIONSHIP: *Rogerus rosae* sp. n. exhibits the generic characteristics of cylindrical stoma, cylindrical esophagus with posterior valvular bulb and outstretched amphidelphic gonads. However, it differs from both *R. orientalis* and *R. rajasthanensis* in the absence of cephalic setae (10 cephalic setae present in *R. orientalis* and 4 in *R. rajasthanensis*) and in having only one dorsal tooth at the beginning of the stoma against 3 equal teeth in both *R. orientalis* and *R. rajasthanensis*). It further differs from *R.*

rajasthanensis in not possessing three glandular organs reported by Khera at the base of the esophagus.

Acknowledgment

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Studies on the Parasites of Chiroptera. I. Helminths of Jamaican Bats of the Genera *Tadarida, Chilonycteris,* and *Monophyllus*

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ABSTRACT: Four species of bats collected in Jamaica have been examined for internal parasites. The following helminths were recovered: Trematoda: *Limatulum gastroides* Macy, 1935 from *Chilonycteris macleayi*; *Prosthodendrium* (*P.*) *swansoni* Macy, 1936 from *Tadarida brasiliensis*; *Urotrema scabridum* Braun, 1900 from *C. macleayi* and *T. brasiliensis*; Nematoda: *Capillaria jamaicanensis* sp. n. from *T. brasiliensis*; *Capillaria* spp. from *C. parnelli* and *Monophyllus redmani*; *Histiostrongylus parnelli* sp. n. from *C. parnelli*; and *Litomosoides guiterasi* (Pérez Vigueras, 1934) Sandground, 1934 from *C. parnelli.* The new species are described and figured. Many findings represent new parasite–host records; all are new with respect to geographical distribution.

The helminth fauna of some Central and South American bat species is fairly well known. However, the author is unaware of any published data on the helminths of bats from Jamaica. Several species of bats were recently collected in Jamaica and kindly made available to us by Dr. A. W. F. Banfield, National Museum of Natural Sciences, Ottawa. This collection included specimens of *Tadarida brasiliensis* (Geoffr.); *Monophyllus redmani* Leach; *Chilonycteris parnelli* (Gray); and *C. macleayi* (Gray).

All bats, received frozen, were thawed, examined for external parasites, and dissected to remove the heart, lung, and complete gastrointestinal tract. All tissues were examined in saline using a dissecting microscope. Trematodes were stained with Harris' haematoxylin stain. Nematodes were cleared in an alcohol–phenol solution. Drawings were made with the use of a Zeiss drawing tube. Unless otherwise noted, specimens have been deposited in the Animal Diseases Research Institute Parasite Collection.

Table 1 shows the helminth parasites recovered. New host–parasite records are marked with an asterisk.

Table 1. Helminths collected from Jamaican bats.

Host	Locality	Parasite (* new host record)	Incidence (No. with parasites/ No. examined)
Tadarida brasiliensis	Golden Grove Cave	**Prosthodendrium* (*P.*) *swansoni* Macy, 1936	8/15
		Urotrema scabridum Braun, 1900	4/15
		Capillaria jamaicanensis sp. n.	9/15
Chilonycteris parnelli	Golden Grove Cave	*Capillaria* sp.	1/6
		Histiostrongylus parnelli sp. n.	2/6
		**Litomosoides guiterasi* (Pérez Vigueras, 1934) Sandground, 1934	2/6
	St. Clair Cave	*Capillaria* sp. (see text)	2/6
		**Litomosoides guiterasi* (Pérez Vigueras, 1934) Sandground, 1934	1/6
Chilonycteris macleayi	St. Clair Cave	**Limatulum gastroides* Macy, 1935	8/29
		**Urotrema scabridum* Braun, 1900	4/29
Monophylus redmani	St. Clair Cave	*Capillaria* sp.	1/1

Capillaria jamaicanensis sp. n. (Nematoda) Figs. 1–3

HOST: *Tadarida brasiliensis.*

HABITAT: Stomach.

LOCALITY: Golden Grove Cave.

INCIDENCE: In 9 of 15 hosts. In no case were more than two nematodes found in the same individual.

DESCRIPTION: (based on 4 male and 2 female specimens). Capillariidae. Moderate-sized specimens possessing general characters of the genus. Male: Length 6.5–10 mm; maximum width approximately 50 μ. Esophagus 1,600–2,800 μ long, dividing body in a ratio of 1:2.6. Spicule 1,050–1,650 μ long. Spicular sheath extruded, massive, without spines except for a small number on each of a pair of ventro-lateral subterminal processes. Pre-bursal alae present. Caudal bursa present; supported by a pair of bifid papillae. Female: Length 8–12.5 mm; maximum width approximately 100 μ. Esophagus 2,800–3,060 μ long, dividing body in a ratio of 1:2.8–3.1. Vulva immediately posterior to end of esophagus; cuticular flap present. Typical operculated eggs, 35–40 $\times$ 20–25 μ.

SPECIMENS: Holotype male USNM Helm. Coll. 70752; Allotype female USNM Helm. Coll. 70753; Paratype male USNM Helm. Coll. 70754.

DISCUSSION: Eleven fairly well documented species of *Capillaria sensu latu* are known to occur in bats of the western hemisphere. The Jamaican specimens described herein are associated with that group of capillariids having a proportionately short esophagus and an aspinous spicular sheath (*Aonchotheca* Lopez-Neyra, 1949). Of this group, three are parasitic in bats: *C. cubana* Freitas and Lent, 1937; *C. palmata* Chandler, 1938; and *C. pusilla* Travassos, 1914. The Jamaican specimens differ from them in having a relatively shorter esophagus, in the morphology of the spicular sheath, and length of the spicule. In none of these three does the spicular sheath show a lobed distal end with minute spines as in *C. jamaicanensis* sp. n. The spicule of *C. jamaicanensis* sp. n. is approximately ⅓ the length of that of *C. cubana*; 2 $\times$ the length of that of *C. pusilla*; and only slightly longer than that of *C. palmata.*

C. jamaicanensis sp. n. differs from *C. pulchra* Freitas, 1934, the only other capillariid described from *T. brasiliensis*, in the morphology of the spicular sheath; the minute spines being limited to the small terminal process.

Capillaria sp. Figs. 4 and 5

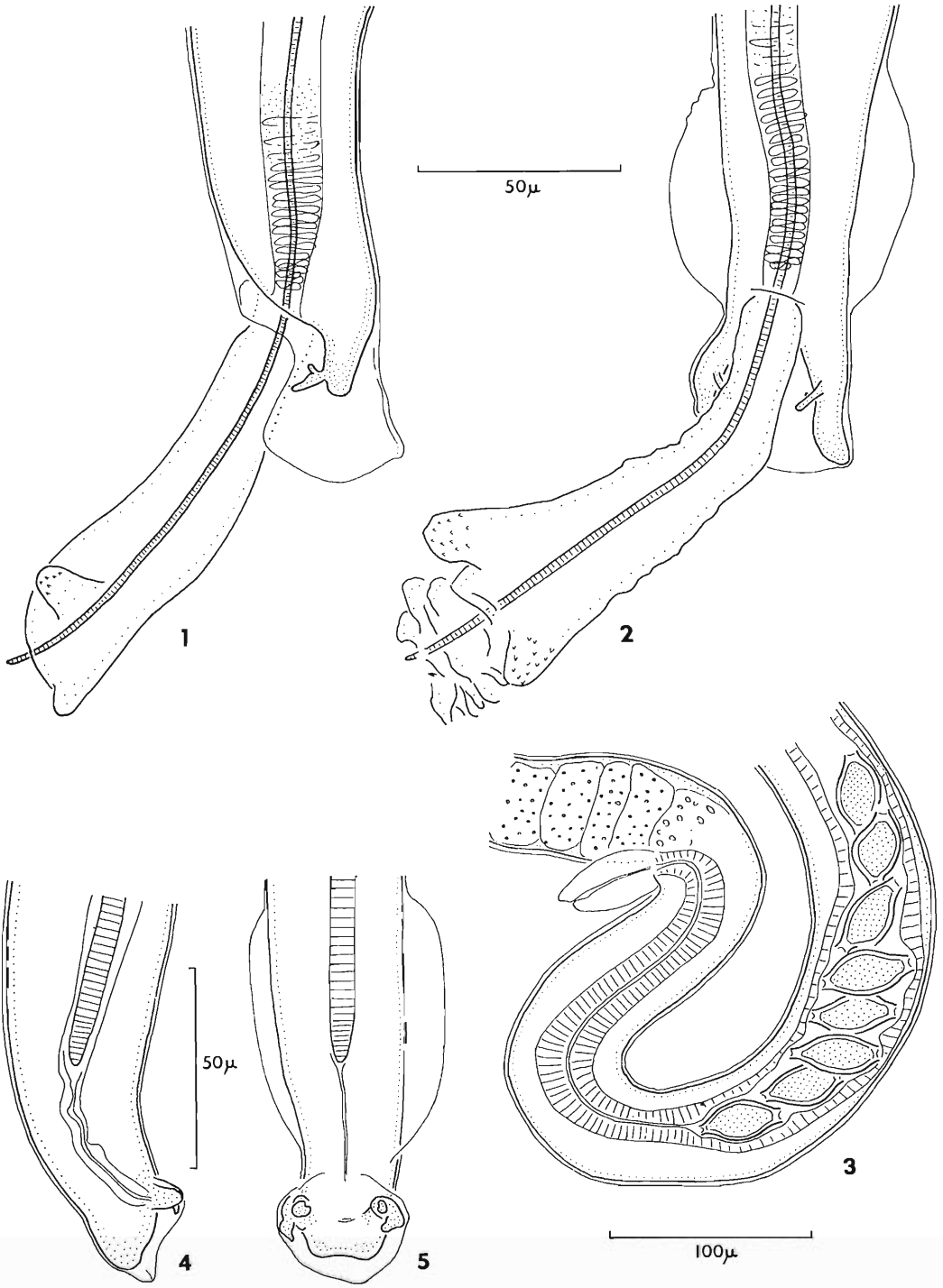
HOST: *Chilonycteris parnelli.*

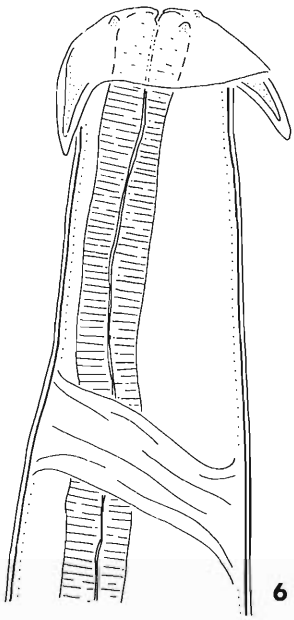
HABITAT: Stomach.

→

Figures 1–3. ***Capillaria jamaicanensis*** **sp. n. Figs. 1, 2. Caudal region of male. Fig. 3. Vulvar region of female.**

Figures 4, 5. ***Capillaria*** **sp. from** ***Chilonycteris parnelli*****; caudal region of male.**





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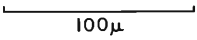

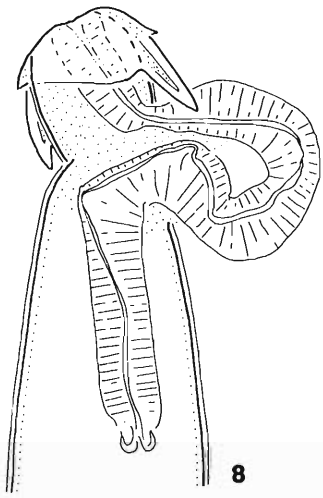

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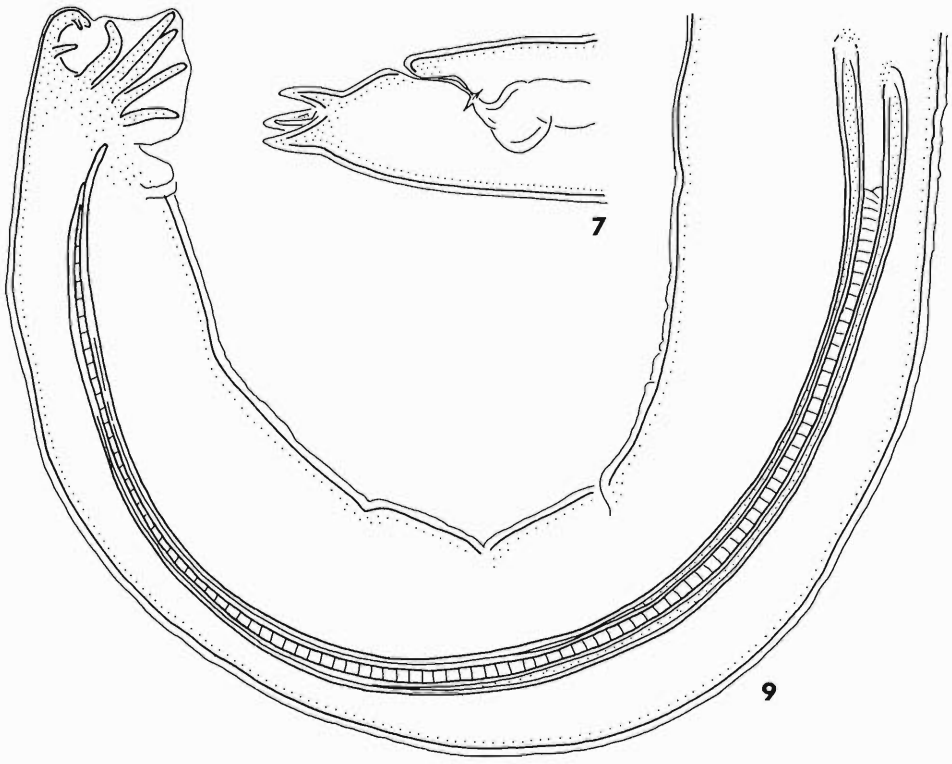

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LOCALITY: St. Clair Cave.

INCIDENCE: In 2 of 6 hosts.

DESCRIPTION: (based on 1 female and the caudal extremity of 1 male). Capillariidae. Female: 9.3 mm long; maximum width 100 μ. Esophagus 3,350 μ long; dividing body in a ratio of 1:1.8. Vulva immediately posterior to end of esophagus; cuticular formations apparently absent. Anus subterminal. Male: prebursal alae present. Small caudal bursa supported by one pair of bifurcating papillae. Spicule 652 μ long. Spicular sheath not spined.

DISCUSSION: Since only a single female and the caudal portion of a single male was recovered, it would be unwise to assign these specimens definitely to a nominate species. However, the dimensions of the female, the male spicule, and the morphology of the caudal bursa and supporting papillae are reminiscent of *C. martinezi* Caballero, 1942 from the stomach of the Mexican bat *Natalus mexicanus*.

Histiostrongylus parnelli sp. n. (Nematoda) Figs. 6–9

HOST: *Chilonycteris parnelli*.

HABITAT: Small intestine.

LOCALITY: Golden Grove Cave.

INCIDENCE: In 2 of 6 hosts.

DESCRIPTION: (based on 1 male and 1 female). Trichostrongylidae: Spinostrongylinae. Cephalic extremity with a cuticular "umbrella" with one pair of large, posteriorly directed spines. Cervical region behind "umbrella" without spines or spinelets. Lateral body alae absent. Buccal cavity small, with a pair of small blunt teeth present. Male: Length 4 mm; maximum width 135 μ. Spicules long, thin, similar, with small alae extending almost their complete length; left spicule 790 μ, right spicule 820 μ long. Gubernaculum absent. Bursa small. Lateral rays longest; posterolateral divergent from others. Dorsal ray curved ventrally. Externodorsal originating from base (?) of dorsal. Ventral rays divergent. Small genital cone present. Female: Length 4.05 mm; maximum width 160 μ. Esophagus cylindrical, 430 μ long. Nerve ring 200 μ from anterior extremity. Vulva salient, without cuticular inflations, 1.8 mm from posterior extremity. Uteri divergent; eggs *in utero* 95–100 × 50–60 μ. Tail 50 μ long; with 1 dorsal and 2 subventral large cuticular spines surrounding a thin terminal filamentous spine.

SPECIMENS: Holotype male USNM. Helm. Coll. 70755, allotype female USNM Helm. Coll. 70756.

DISCUSSION: *Histiostrongylus* Molin, 1861 has heretofore contained but a single species, *H. coronatus* Molin, 1861 found in the following bats: *Phyllostoma discolor* in Brazil (Molin, 1861); *Phyllonycteris poeyi* in Cuba (Pérez Vigueras, 1941; Baruš and Valle, 1967); and *Chilonycteris fuliginosa torrei* in Cuba (Baruš and Valle, 1967). The specimens described herein as *H. parnelli* sp. n. are placed in this genus pending further findings and subsequent descriptions. They are similar to *H. coronatus* in having an umbrella-shaped cephalic hood supporting large, posteriorly-directed spines: two in the case of *H. parnelli* sp. n. and numerous in *H. coronatus*. A small buccal cavity contains two teeth (*H. parnelli* sp. n.) or three teeth (*H. coronatus*) (Pérez Vigueras, 1941). The tail of the female is similar in both species. The spicules of the Jamaican species are simple, similar, undivided, and reminiscent of the type found in various Nematodirinae genera; those of *H. coronatus* are divided distally. A gubernaculum, present in *H. coronatus*, is absent in *H. parnelli* sp. n. The structure of the bursa of the Jamaican specimen is difficult to interpret, particularly with respect to the morphology of the dorsal ray. However, it appears to be similar to that found in *H. coronatus*.

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Figures 6–9. ***Histiostrongylus parnelli*** **sp. n. Fig. 6. Anterior region of female. Fig. 7. Caudal region of female. Fig. 8. Anterior region of male. Fig. 9. Posterior region of male.**

Studies on Helminths of North Dakota. I. Two New Monogenetic Trematodes of the Genus *Gyrodactylus* from Percid Fishes and a Redescription of *G. etheostomae* Wellborn and Rogers, 1967

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ABSTRACT: Two new species of *Gyrodactylus* are described from percid fishes in North Dakota: *G. schmidti* from the walleye, *Stizostedion vitreum* (Mitchill); and *G. mizellei* from *S. vitreum* and the sauger, *S. canadense* (Smith). The finding of *G. etheostomae* Wellborn and Rogers, 1967, on the mud darter, *Etheostoma asprigene* (Forbes), from North Dakota constitutes new host and locality records for this trematode; *G. etheostomae* is redescribed.

The monogenetic trematode fauna of North Dakota is poorly known. The first report was that of Ikezaki and Hoffman (1957) who described *Gyrodactylus eucaliae* from the five-spined stickleback, *Eucalia inconstans* (Kirtland). Mizelle and Kritsky (1967b) described *G. lacustris* and recorded *G. hoffmani* Wellborn and Rogers, 1967, from the fathead minnow, *Pimephales promelas* Rafinesque. The only other record was the description of *G. nebulosus* from the brown bullhead, *Ictalurus nebulosus* (LeSueur), by Kritsky and Mizelle (1968).

In the present paper two new species of *Gyrodactylus* are described from game fishes, and a third species, *G. etheostomae* Wellborn and Rogers, 1967, is redescribed.

Methods and Materials

The sauger, *Stizostedion canadense* (Smith), and walleye, *S. vitreum* (Mitchill), were collected by hook and line from the Garrison Dam tailrace on the Missouri River near Riverdale (Mercer Co.), North Dakota, during the fall of 1970. The mud darter, *Etheostoma asprigene* (Forbes), was seined from the Snake Creek Embankment of the Garrison Reservoir near Coleharbor (McLean Co.), North Dakota, in the fall of 1967. Methods for treatment of hosts and preparation and study of their parasites were employed as given by Mizelle and Kritsky (1967a). Several parasites were stained with Ehrlich's hematoxylin for differentiation of internal anatomy. Measurements are in microns. Paratypes are in the authors' collections.

Gyrodactylus mizellei sp. n. (Figs. 1–3)

HOSTS: Sauger, *Stizostedion canadense* (Smith) (type), and walleye, *S. vitreum* (Mitchill).

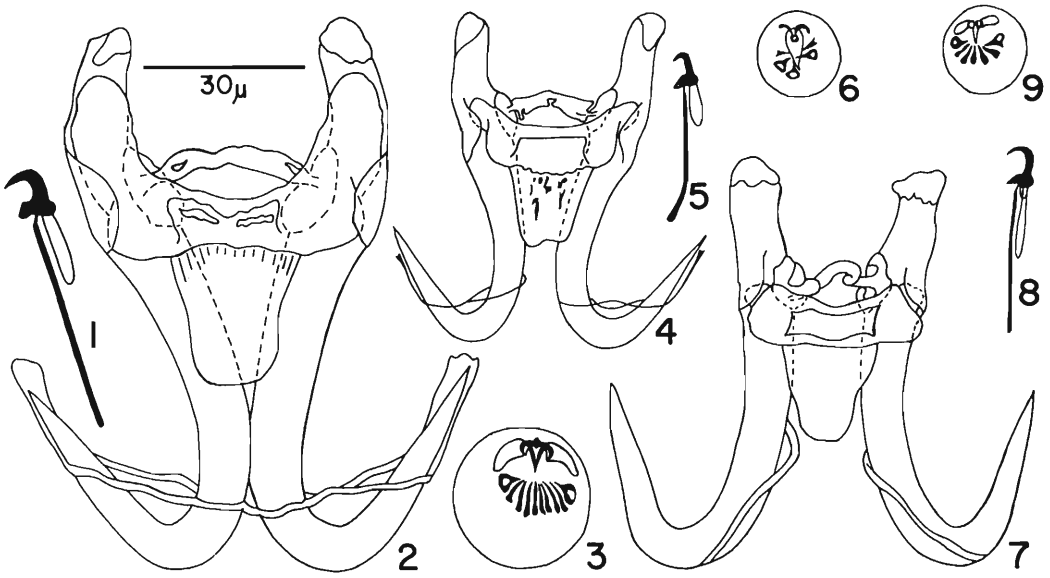
LOCATION ON HOST: External surface.

SPECIMENS STUDIED: 16 from *S. canadense*, 6 from *S. vitreum*; in the description, measurements of specimens from *S. vitreum* are given in quotes subsequent to those from the type host.

HOLOTYPE: USNM Helm. Coll. (No. 71657).

Description

With characters of the genus as emended by Mizelle, Whittaker, and McDougal, 1969. Length 477 (378–648) "626 (529–692)," greatest width 126 (97–155) "135 (108–173)" in posterior half. Cephalic lobes moderate to absent, each (or area) with conspicuous dorsal spike sensilla and subterminal cavity. Head organs inconspicuous, longitudinally striated, form small papillae in cephalic-lobe cavity. Anterior pharyngeal bulb 32 (28–39) "34 (30–37)" wide, papillae elongate; posterior bulb 37 (30–45) "41 (33–54)" wide; pharynx 39 (30–45) "46 (42–51)" long; intestinal crura blind. Haptor ovate, 126 (108–157) "135 (97–152)" long, 132 (86–173) "167 (151–195)" wide; hook distribution extrahamular. Anchor 94 (86–104) "98 (89–105)" long, root variably bent, fold and knob well developed, base 16 (12–20) "16 (14–18)" wide. Superficial bar 51 (39–63) "51 (47–54)" long, ends extend to near tip of anchor bases, shield narrow with



Figures 1–3. ***Gyrodactylus mizellei*** **sp. n. 1, Hook. 2. Anchor and bar complex. 3, Cirrus. Figures 4–6.** ***G. etheostomae*** **Wellborn and Rogers, 1967. 4, Anchor and bar complex. 5, Hook. 6, Cirrus. Figures 7–9.** ***G. schmidti*** **sp. n. 7, Anchor and bar complex. 8, Hook. 9, Cirrus.**

faint proximal lines. Deep bar inflated subterminally, 34 (30–40) "30 (28–34)" long. Marginal hook 38 (36–40) "38 (37–39)" long, shank uniform. Hooklet 8 (7–9) "7 or 8" long, shaft and recurved point robust, toe blunt, base concave with small globose heel and distinct shelf; filamentous hooklet (FH) loop ⅔ to ½ length of shank. Ovary ovate, saccate, ventral, postuterine, usually containing a large ovum; embryo occasionally inverted. Subhemispherical testis abuts posterodorsal ovarian wall. Cirrus sinistral, postpharyngeal, with 8 to 10 spinelets; diameter 16 (13–19) "19 (17–20)." Specimens with maximum of three embryos; development of haptoral parts normal; temporal development early (see Mizelle and Kritsky, 1967a).

Remarks

Gyrodactylus mizellei sp. n. resembles *G. hoffmani* Wellborn and Rogers, 1967, in the shape of the anchors. However, they are easily distinguished by the morphology of the haptoral bars and hooks. This species is named for Dr. John D. Mizelle, Sacramento State College, Sacramento, California.

Gyrodactylus etheostomae Wellborn and Rogers, 1967 (Figs. 4–6)

HOST: Mud darter, *Etheostoma asprigene* (Forbes).

PREVIOUSLY REPORTED HOST AND LOCALITY: Orangebelly darter, *E. radiosum* (Hubbs and Black); Warm Fork of Spring River, National Fish Hatchery, Mammoth Spring (Fulton Co.), Arkansas (Wellborn and Rogers, 1967).

LOCATION ON HOST: External surface.

SPECIMENS STUDIED: 7; a specimen was deposited in USNM Helm. Coll. (No. 71659).

Redescription

With characters of the genus as emended by Mizelle et al., 1969. Length 443 (356–480), greatest width 115 (87–135) in anterior trunk. Cephalic lobes conspicuous, each with dorsal spike sensilla as described in *Gyrodactylus* sp. by Lyons (1969); head organs poorly developed; conspicuous cephalic glands posterolateral to pharynx. Anterior pharyngeal bulb 31 (27–37) wide, disc-shaped; papillae inconspicuous. Posterior bulb subovate, 31 (28–35) wide; pharynx 30 (28–32) long; intestinal crura

blind. Haptor subovate to subhemispherical, 79 (68–86) long, 80 (64–100) wide; hook distribution extrahamular. Anchor 47 or 48 long, with conspicuous fold and knob; base 7 or 8 wide; filament conspicuous. Superficial bar 25 (23–26) long, ends directed anterolaterally; shield short, posterior margin indented. Deep bar with subterminal enlargements and deep median notch, 14 (11–16) long. Marginal hook 24 (23–25) long, proximal portion of shank enlarged; hooklet 5 or 6 long, point recurved, shaft straight, base with shelf, globose heel and blunt toe; FH loop ⅓ of shank length. Ovary indistinct; uterus with 1 to 3 embryos. Testis ovate, postovarian. Cirrus 11 (10–12) in diameter, dextral with 3 to 5 spinelets, laterals larger. Embryos insufficient to determine sequential development of haptoral parts and temporal development of consecutive embryos.

Gyrodactylus schmidti sp. n. (Figs. 7–9)

HOST: Walleye, *Stizostedion vitreum* (Mitchill).

LOCATION ON HOST: External surface.

SPECIMENS STUDIED: 5.

HOLOTYPE: USNM Helm. Coll. (No. 71658).

Description

With characters of the genus as emended by Mizelle et al., 1969. Length 499 (378–551), greatest width 126 (108–173) near midlength or in posterior half. Cephalic lobes moderate, each with dorsal spike sensilla; head organs inconspicuous, form small papillae in cephalic-lobe cavity. Anterior pharyngeal bulb 29 (27–32) wide, papillae not observed; posterior bulb 34 or 35 wide; pharynx 29 (24–34) long; intestinal crura apparently blind. Haptor subovate, 103 (86–119) long, 108 (97–119) wide; hook distribution extrahamular. Anchor 62 (61–63) long, superficial root variable, fold extensive, shaft gently curved, base 12 (10–14) wide. Superficial bar 28 (27–29) long, anterolateral projections short, shield tapered. Deep bar vermiform, variably bent, 18 (16–19) long. Hook 28 (27–29) long, shank uniform; hooklet 6 or 7 long, point recurved, shaft straight, base with shelf, blunt toe and globose heel; FH loop ½ length of shank; secondary filamentous hooklet (SFH) loop short, indistinct. Ovary postuterine, usually with large ovum. Testis postovarian, irregular. Cirrus 12 or 13 in diameter, sinistral, with 7 spinelets, laterals slightly larger. A maximum of two embryos in uterus; development of haptoral parts normal (see Mizelle and Kritsky, 1967a) except anchor points form after hook shanks and hooklet base; embryos insufficient for determination of temporal development.

Remarks

The closest relative of this species appears to be *Gyrodactylus crysoleucas* Mizelle and Kritsky, 1967, from which it differs in the morphology of the haptoral armament and cirrus. The species is named for Dr. G. P. Schmidt, University of Northern Colorado, Greeley, Colorado.

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Activity of Levamisole, Pyrantel Tartrate, and Rafoxanide Against Two Thiabendazole-tolerant Isolates of *Haemonchus contortus*, and Two Species of *Trichostrongylus*, in Sheep

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ABSTRACT: The activity of 3 of the newer anthelmintics against two local thiabendazole-tolerant isolates of *Haemonchus contortus* (BPL-2 and AH-2), as well as against *Trichostrongylus axei* and *T. colubriformis*, was compared in experimentally infected lambs, using the method of the controlled anthelmintic test. Twenty of the 40 lambs on test were each given *per os* 5,000 infective larvae of the BPL-2 isolate of *H. contortus*, and the other 20 were given 5,000 infective larvae of the AH-2 isolate. Also, each lamb was given 18,000 *T. axei* and 18,000 *T. colubriformis* infective larvae. Single doses of the test drugs were given to the appropriate groups of lambs 21 days postinfection, and all lambs were killed for worm counts 6 to 7 days later. Levamisole was given at 8mg/kg of body weight, pyrantel tartrate at 25 mg/kg, and rafoxanide at 5 mg/kg. All 3 anthelmintics were markedly effective (99–100%) against the 2 isolates of *H. contortus*. Levamisole was very effective also (95–99%) against the 2 species of *Trichostrongylus*. Pyrantel tartrate was highly effective against *T. axei* (99%), but substantially less effective (67%) against *T. colubriformis*. Rafoxanide showed no activity against *Trichostrongylus* spp.

Previously, we reported (Colglazier, Kates, and Enzie, 1970) that 2 isolates of *H. contortus* from sheep showed tolerance to the standard therapeutic dose of thiabendazole, and that 1 isolate (AH-2) was more tolerant than the other (BPL-2). The literature on thiabendazole-resistant strains of *H. contortus* has been summarized (Smeal et al., 1968; Theodorides, Scott, and Laderman, 1970; Colglazier et al., 1970). In related observations, Kates et al. (1971) and Theodorides et al. (1970) found that thiabendazole-tolerant strains of *H. contortus* were also resistant to parbendazole, another benzimidazole compound.

Because of the apparent widespread occurrence of thiabendazole- and parbendazole-tolerant strains of *H. contortus*, it seemed desirable to determine the efficacy of some of the newer anthelmintics against 2 local thiabendazole-tolerant isolates. It seemed appropriate also to determine concurrently the action of these compounds against 2 common pathogenic species of *Trichostrongylus*.

The test drugs were levamisole, pyrantel tartrate, and rafoxanide. All have shown significant activity against *H. contortus*, but none has been tested extensively against thiabendazole-tolerant strains of the parasite. The significant literature on levamisole was recently summarized by Kates et al. (1971), and that on pyrantel tartrate against gastrointestinal nematodes of sheep by Cornwell and Jones (1969). There is only one report on the activity of rafoxanide against *H. contortus* (Egerton, Yakstis, and Campbell, 1970). This drug is also active as a fasciolicide (Campbell, Ostlind, and Yakstis, 1970).

Materials and Methods

Protocol of the controlled anthelmintic test

The 40 Polled Dorset lambs were raised parasite-free except for insignificant infections of *Strongyloides papillosus* and coccidia. At the start of the experiment the lambs had a mean age of about 8 months and a mean weight of about 40 kg. An equal number of wether and female lambs were used, and the two sexes were divided as equally as possible among the experimental groups. Lambs were also allocated to the experimental groups so that the group mean weights were approximately the same.

Each of 20 lambs (divided into 4 equal groups) was given by mouth 5,000 infective larvae of the BPL-2 isolate of *H. contortus* and 18,000 *T. axei* and 18,000 *T. colubriformis* infective larvae, a total of 41,000 larvae per lamb. The other 20 lambs were grouped and infected similarly with the AH-2 isolate of *H. contortus* and the 2 species of *Trichostrongylus*.

Table 1. Activity of levamisole, pyrantel tartrate, and rafoxanide against experimental gastrointestinal nematode infections in sheep.

Parasites (Lambs/group)	Larvae/lamb ± CI (95%)[1]	Unmedicated Controls Average and (Range)	Average worms at necropsy and efficacy: Levamisole (8 mg/kg) Average	Levamisole (8 mg/kg) Efficacy (%)	Pyrantel tartrate (25 mg/kg) Average	Pyrantel tartrate (25 mg/kg) Efficacy (%) calculated	Rafoxanide (5 mg/kg) Average	Rafoxanide (5 mg/kg) Efficacy (%)
H. contortus								
BPL-2 Isolate (5 lambs)	5,000 ± 161	1,273 (15–2,700)	<1 (0–1)	99	<1 (0–2)	99	<1 (0–1)	99
AH-2 Isolate (5 lambs)	5,000 ± 159	1,394 (3–2,549)	0	100	0	100	<1 (0–1)	99
T. axei (10 lambs)	18,000 ± 728	10,705 (6,883–16,442)	500 (0–2,160)	95	152 (0–260)	99	12,908 (10,120–16,100)	0
T. colubriformis (10 lambs)	18,000 ± 1,032	15,605 (9,780–17,700)	182 (20–540)	99	5,155 (80–6,720)	67	13,658 (7,960–17,440)	13

[1] CI = Confidence Interval = Standard error × 2.26 (t @ 9 degrees of freedom).

The origin of the two isolates of *H. contortus* was previously given by Colglazier et al. (1970). The larvae of *Trichostrongylus* spp. were of bovine origin. One group of 5 lambs for each isolate of *H. contortus* served as unmedicated controls, and each of the other 3 groups was dosed with 1 of the 3 test drugs. Because all lambs were given equivalent infections of the two species of *Trichostrongylus*, the control and medicated groups for these species contained 10 lambs (Table 1).

Before infecting the lambs, the freshly harvested larvae were quantitated by making 10 representative counts of each larval suspension. From these counts the standard errors and 95% confidence intervals were calculated (Table 1). Appropriate quantities of the larval suspensions for each of the 3 nematode species were combined and given as a single dose to each lamb.

During the course of this trial, the several groups of lambs were maintained in separate isolation pens and fed a standard maintenance ration. Single therapeutic doses of the appropriate anthelmintics were given to the test lambs 21 days postinfection as indicated below and in Table 1. All lambs were killed for worm counts 6 to 7 days later, using standard techniques as described by Colglazier et al. (1970) and Kates et al. (1971).

Anthelmintics used[1]

Levamisole, *l*-2,3,5,6-tetrahydro-6-phenylimidazo (2,1-*b*) thiazole hydrochloride: pure chemical for experimental use; American Cyanamid Co., Princeton, New Jersey: 8 mg/kg of body weight.

Pyrantel tartrate, trans-1-methyl-1,4,5,6-tetrahydro-2-[(2-thienyl-vinyl)]-pyrimidine tartrate: pure chemical for experimental use; Chas. Pfizer & Co., Terre Haute, Indiana: 25 mg/kg of body weight.

Rafoxanide [3,5-diiodo-3′-chloro-4′-(*p*-chlorophenoxy)-salicylanilide]: 3.035% aqueous suspension for experimental use; Marck & Co., Rahway, New Jersey: 5 mg/kg of body weight.

Results and Discussion

The data (Table 1) show that all 3 anthelmintics under test were highly effective (99–100%) against both thiabendazole-tolerant iso-

[1] Mention of products used in this study does not constitute endorsement by the USDA.

lates of *H. contortus*. This finding contrasts markedly with the results obtained against these isolates with thiabendazole (Colglazier et al., 1970). In the latter report, aggregate data from 3 trials showed that at the standard dose rate of 50 mg/kg of body weight, thiabendazole removed only 67% of the BPL-2 isolate of *H. contortus* and only 39% of the AH-2 isolate from experimentally infected lambs. It is apparent, therefore, that the 3 newer drugs used in the present trial should prove useful in treating sheep infected with these, and perhaps other, thiabendazole-resistant strains of *Haemonchus*.

Rafoxanide is primarily a fasciolicide (Campbell et al., 1970), although it has activity also against *H. contortus* (Egerton et al., 1970). Our data show that this compound was ineffective against *T. axei* and *T. colubriformis* (0 and 13%, respectively); thus, it has only limited usefulness against gastrointestinal nematodes of sheep. Levamisole showed excellent activity against both species of *Trichostrongylus* in this trial (95 and 99%), which confirms numerous reports in the literature. Pyrantel tartrate was highly active (99%) against *T. axei*, but it was much less effective (67%) against the intestinal species, *T. colubriformis*. Our results with pyrantel tartrate against *T. colubriformis* did not compare favorably with those reported by other investigators. Cornwell (1966), using dosages of 25 and 45 mg/kg, obtained more than 90% removal of adult worms; and at the 25-mg dose level, Gibson and Parfitt (1968) reported 80% removal of 28-day-old infections of this species.

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Freshwater Larval Trematodes. XXVIII. Three New Species of Cercariae

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ABSTRACT: *Cercaria barceloica*, of gymnocephalic group, *C. farakhanweri* and *C. paracumanensis*, xiphidiocercariae of microcotylous group, from the snails, *Pomacea glauca*, *P. urceus* and *Marisa cornuarietis*, in different regions of Venezuela are described. A comparison is made with related species.

A gymnocephalic cercaria, which later proved to be a new species and was named *Cercaria barceloica*, was easily confused with the two other Venezuelan cercariae, *C. macarapanensis* Nasir and Acuña (1966) and *C. sanlorenzensis* Nasir and Acuña (1964). Closer examination, especially of the flame cell system, revealed the presence of two distinct species. The same was true in case of two xiphidiocercariae, *C. farakhanweri* and *C. paracumanensis*, which could be mistaken for *C. cumanensis* Nasir (1965). *Cercaria paracumanensis* is a unique microcotylous form in having a group of three flame cells with anterior and posterior collecting tubules on each side of body, while the other representatives of this group are characterized by paired flame cells.

For measurements, freshly emerged cercariae, 12 of each species, were mounted in a drop of water, under a coverglass, and excess liquid was absorbed with a piece of blotting paper until their activities were curtailed. They were then heat-fixed by placing them, for a minute or so, near an incandescent lamp. This method gave the most uniform results. The diagrams have been made with the aid of camera lucida; the measurements are in millimeters.

Results

A. Gymnocephalic Group

Cercaria barceloica sp. n. (Fig. 1, 1a)

HOST: *Pomacea glauca* (L.).

LOCALITY: Río Barcelo, en route to Güiria, Edo. Sucre, Venezuela.

DESCRIPTION: Body spinose, without eyespots. Tail aspinose, without finfold. Suckers equal in diameter. Oral sucker with a row of papillae around its periphery, and another row around oral orifice. Ventral sucker surrounded by a muscular ring, without papillae, located posterior to equatorial line of body; a row of spines interior to acetabular periphery. Prepharynx and pharynx well developed. Esophagus extending to ventral sucker. Intestinal ceca short, not reaching beyond midlevel of ventral sucker. Six apertures, at anterior end of body, leading into six penetration ducts, which could not be traced to corresponding glands. Cystogenous glands with rhabditiform contents, mostly distributed in lateral areas. Numerous glands, with granular contents, limited to preacetabular region, between ascending limbs of excretory system. Excretory system as shown in Fig. 1a; secondary excretory tubes ciliated, dividing posterior to equatorial level of ventral sucker; flame cell formula $2\,[(3+3+3)+(3+3)] = 30$. Genital rudiments represented by two cellular masses, one anterior and other posterior to acetabulum. Measurements: body 0.210–0.266 by 0.140–0.238; tail 0.168–0.252 by 0.056–0.140; oral sucker 0.056–0.088 in diam.; ventral sucker 0.056–0.092 in diam.; prepharynx 0.008–0.048 long; pharynx 0.020–0.032 by 0.015–0.025.

B. Xiphidiocercariae

Cercaria farakhanweri sp. n. (Fig. 2, 2a, 2b)

HOST: *Pomacea urceus* (L.).

LOCALITY: Tucupido, Edo. Guarico. Venezuela.

DESCRIPTION: Body spinose. Tail aspinose, without finfold, considerably subterminal, with-

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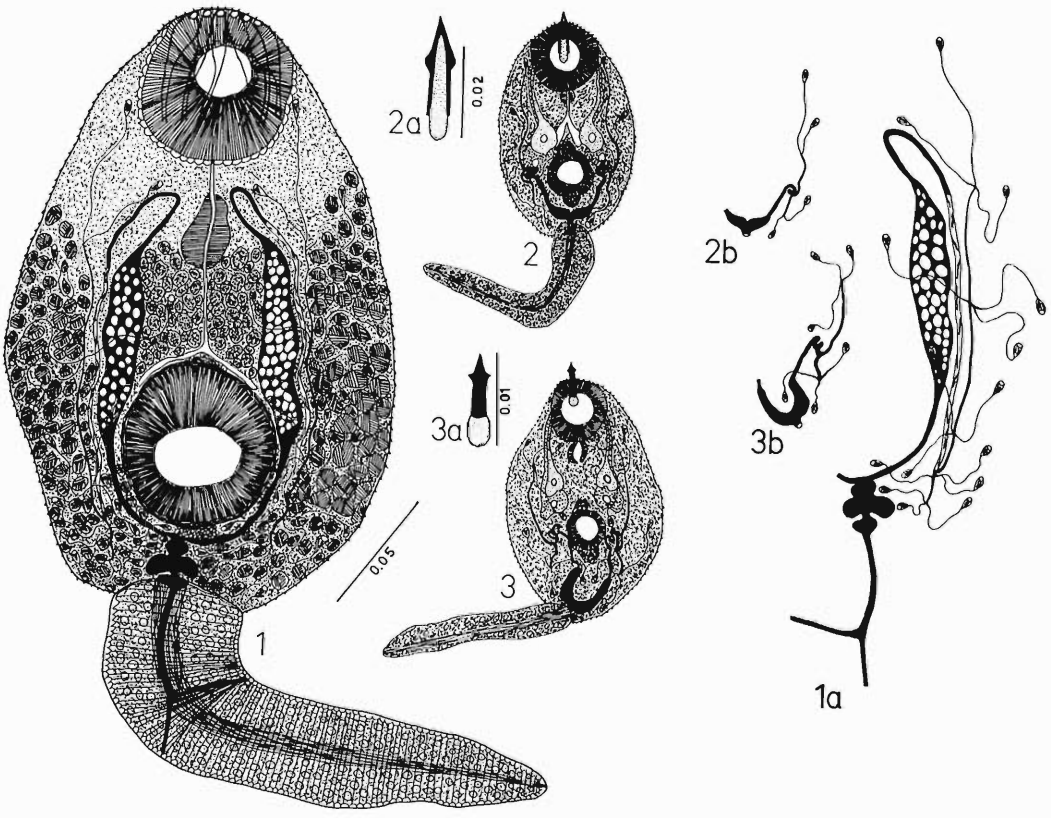

Figures 1–3b. 1. *Cercaria barceloica* sp. n. 1a. Excretory system drawn on one side only. 2. *Cercaria farakhanweri* sp. n. 2a. Stylet, note the presence of a basal bulb. 2b. Details of excretory system on one side only. 3. *Cercaria paracumanensis* sp. n. 3a. Stylet, note the presence of a basal bulb. 3b. Details of excretory system on one side only.

All three species with corresponding excretory details drawn to the same scale.

out caudal pockets. Prepharynx absent. Pharynx small. Esophagus slightly longer than pharynx, not extending to ventral sucker. Intestinal ceca considerably more dilated than esophagus, hardly reaching ventral sucker. Penetration glands in two pairs, pre- and paracetabular: anterior with finely granular contents; posterior pair coarsely granular; two penetration ducts on each side of body. Stylet with a basal bulb, basal part not reinforced, Fig. 2a. Excretory system as shown in Fig. 2b; main excretory tubes dividing at equatorial level of ventral sucker; flame cell formula $2[(2) + (2)] = 8$; no caudal excretory duct. Development in sausage shaped sporocysts. Measurements: body 0.052–0.099 by 0.052–0.064; tail 0.040–0.096 by 0.012–0.024; stylet including basal bulb 0.024–0.028 long, 0.006–0.008 wide at shoulder; oral sucker 0.023–0.036 in diam.; ventral sucker 0.012–0.020 in diam.; pharynx 0.008–0.016 in diameter.

Cercaria paracumanensis sp. n. (Fig. 3, 3a, 3b)

HOST: *Marisa cornuarietis* (L.).

LOCALITY: Hacienda Montalban, near Cumanacoa, Edo. Sucre, Venezuela.

DESCRIPTION: Body spinose. Tail aspinose, considerably subterminal, without a finfold. Shape of stylet as shown in Fig. 3a, with a basal bulb. Oral sucker larger than ventral.

Prepharynx absent. Pharynx present. Esophagus and ceca absent. Penetration glands in two pairs, mostly preacetabular: anterior pair finely granular; posterior pair coarsely granular; two penetration ducts on each side of body. Excretory system as shown in Fig. 3b; excretory vesicle V- or U-shaped; main excretory tubes dividing posterior to or in postequatorial region of ventral sucker; flame cell formula 2 [(3) + (3)] = 12. Measurements: body 0.084–0.096 by 0.052–0.060; tail 0.072–0.080 by 0.012–0.020; stylet including basal bulb 0.010–0.014 by 0.002–0.006; oral sucker 0.028–0.036 in diam.; ventral sucker 0.016–0.020 in diam.; pharynx 0.008–0.016 in diameter.

Discussion

The freshwater cercariae, like *Cercaria barceloica*, lacking a caudal finfold, collar spines, and eyespots, but characterized with rhabditiform contents of cystogenous glands, and in which the intestinal ceca fail to extend beyond the posterior margin of the ventral sucker are: *Cercaria macarapanensis* Nasir and Acuña (1966), flame cell formula 2 [(2 + 2 + 2) + (2 + 2 + 2)] = 24, *C. sanlorenzensis* Nasir and Acuña (1964), 2 [(2 + 2 + 2) + (2 + 2 + 2)] = 24, *Echinochasmus zubedakhaname* Nasir and Díaz (1968), 2 [(2) + (2 + 2)] = 12, and *Stephanoprora paradenticulata* Nasir and Rodriguez (1969), 2 [(3 + 3 + 3) + (3 + 3 + 3)] = 36. All of these species are readily separated by having a different flame cell formula from that of *C. barceloica*, which has 2 [(3 + 3 + 3) + (3 + 3)] = 30. Also different are the pattern of the excretory tubules, the extent of the intestinal ceca in relation to the ventral sucker, and the presence or absence of the papillae or spines on suckers. *C. granocutis* Pike (1968), and *C. llangorsensis* Probert (1965) are very similar to *C. barceloica* in having a total number of 30 flame cells, but their intestinal ceca extend to the posterior end of the body in contrast to those found in *C. barceloica*. It may be mentioned here that in these two species the details of the excretory tubules are unknown, thus a detailed comparison is not possible.

Gupta and Taneja (1968) described a larval form of the "Agilis" group as *Cercaria* (*Gymnocephalous*) sp. of Thapar and Tandon, 1952, from *Lymnaea accuminata*, in Patiala, Panjab, India, and without experimental evidence considered it as the cercaria of *Fasciola gigantica* Cobbold, 1855. There are no details of its excretory system. The contents of the cystogenous glands are rhabditiform, but the intestinal ceca are shown only slightly beyond the esophagus. Porter (1920; 1938), as a result of experimental studies, has clearly shown the intestinal ceca in the same species as extending to the posterior end of the body. Another confusing form, also considered without experimental evidence as the larva of *F. gigantica*, has been introduced by Singh and Malaki (1963), from *Lymnaea* (*Pseudosuccinea*) *accuminata*, in India. There is no description, but as shown by them (Fig. 4) the esophagus is only partly represented and the contents of the cystogenous glands are granular. One wonders about the identity of this species, because the cercaria of *F. gigantica* is provided with rhabditiform contents of the cystogenous glands, and the intestinal ceca extend to the posterior end of the body. Kuntz (1957) worked out the embryonic development of the excretory system of a cercaria which was also regarded, again without experimental evidence, as the larva of *F. gigantica*, from *Lymnaea natalensis caillaudi*, in Giza, Cairo, Egypt. Although not mentioned in the text the cystogenous glands are shown in the diagrams to be granular.

Cercaria cellulosa Looss, 1900, (Wesenberg, Lund, 1934), *Cephalouterina dicamptodoni* Senger and Macy, 1953, (Anderson, Martin, and Pratt, 1966), *Cercaria cumanensis* Nasir, 1965, *C. cystorhysa* Miller, 1935, (Miller, 1936), *C. gingindhlovia* Porter, 1938, *C. gregaria* O'Roke, 1917, *C. homocotylea* Nasir and Acuña, 1966, *C. indicae* LVII Sewell, 1922, *C. meniscadena* Miller, 1935, (Miller, 1936), *C. minuta* Probert, 1965, *C. naukuchiensis* Malaki and Singh, 1962, *C. parapaucadena* Porter, 1938, *C. pugnax* La Valette, 1854, (Ginetsinskaya and Dobrovolski, 1968), *C. pusilla* Looss, 1900, (Wesenberg-Lund, 1934), and *C. sansoucia* Porter, 1938, are the other microcotylus xiphidiocercariae furnished with two pairs of penetration glands as in *C. farakhanweri*, but only *C. cystorhysa*, *C. meniscadena*, *C. minuta*, *C. naukuchiensis*, *C. pugnax*, and *C. pusilla* have the contents of the penetration glands differentiated. However, none of these species has the same flame cell formula, i.e., 2 [(2) + (2)] = 8 as that in *C. farakhanweri*. This cercaria is

further set apart in the combination of one or more of the following characters: the nature of the contents of the penetration glands, i.e., which of the two pairs is finely granular, the position of these glands in relation to the ventral sucker, the shape and size of stylet, the nature of gut, the relative size of the suckers, and the shape of excretory vesicle.

Cercaria cellulosa, *C. cumanensis*, *C. gingindhlovia*, *C. homocotylea*, *C. indicae* LVII, *C. parapaucadena*, and *C. sansoucia* have a flame cell formula $2[(2) + (2)] = 8$ and, thus, are more closely allied to *C. farakhanweri* but the contents of their penetration glands are not differentiated into finely and coarsely granular inclusions. *C. chacaracualensis* Nasir and Acuña, 1966, parasite of *Marisa cornuarietis* (L.), from Quebrada de Chacaracual, in Edo. Sucre, Venezuela, also possesses two pairs of penetration glands (without differentiated contents), but to the glands of each pair is associated a more or less pyriform structure without any apparent inclusions and the flame cell formula is $2[(2 + 2) + (2 + 2)] = 16$. *C. cyclica* Miller, 1936, also a microcotylous form, is unique in that there is one penetration gland on each side of the ventral sucker leading into a duct which contains a nucleated swollen region. The nucleated region may be interpreted as a gland, thus there are two glands on each side of body. There is also a duct, not associated with any glandular equipment, running from the oral region on one side to the ventral sucker, then continuing again anteriorly on the other side (Miller, 1936, Fig. 52).

Cercaria paracumanensis, another microcotylous larva encountered during the present investigation, is similar to *C. farakhanweri* in having spinose body, the ventral sucker smaller than the oral, the anterior pair of the penetration glands finely granular, the posterior pair coarsely granular, and in the position of these glands in relation to the ventral sucker. On the other hand, *C. farakhanweri* has an esophagus, two short intestinal ceca, and a total number of eight flame cells in contrast to the complete absence of the gut beyond pharynx, a different shape of stylet, and a total of twelve flame cells in *C. parcumanensis*. The latter has been frequently confused with *C. cumanensis* because of the approximate shape and size of its stylet, and the utilization of the same intermediate host, *Marisa cornuarietis*. However, closer examination revealed the presence of two distinct species. *Cercaria cumanensis* has a small esophagus, undifferentiated contents of the penetration glands, and eight flame cells in contrast to the complete absence of the gut beyond pharynx, differentiated contents of the penetration glands, and twelve flame cells of *C. paracumanensis*.

Ahmed and Khan (1967) described a new species of a microcotylous xiphidiocercaria, *Cercaria chilyaensis*, from *Vivipara bengalensis* (Lamarck), in Chilya Lake, Hyderabad, West Pakistan, characterized with two pairs of penetration glands, without differentiated contents, absence of esophagus and ceca, and an oblong excretory vesicle which gives off an anterior duct, bifurcating into the lateral excretory tubes. They also stated "the caudal excretory duct gives off a number of lateral irregular branches. In the tail region the excretory canal does not reach up to the posterior extremity of the tail but ends a little above." It is now well known (Hussey, 1941, and La Rue, 1957) that in the xiphidiocercariae no portion of the excretory system is carried down in the tail. Thus, it is hard to understand how the tail of *C. chilyaensis*, which is a xiphidiocercaria, could possibly have been traversed by a caudal excretory duct! The same authors also added "the cystogenous cells hindered the visibility and any further branching of excretory canals or even the flame cells were not observed." It is surprising that so many cystogenous glands could have been present in a microcotylous larva which is at the simplest organizational level of the xiphidiocercariae. Perhaps the authors were observing, rather inadvertently, a gymnocephalic or an echinostome cercaria which indeed is heavily provided with the cystogenous glands.

Cercaria naukuchiensis Malaki and Singh (1962), a parasite of *Melanoides tuberculatus* (Müller) var. *tigrina* (Hutton), from Naukuchia Tal, Bhimtal, in India, possesses two pairs of penetration glands: one external, smaller, preacetabular pair, with finely granular contents; an internal, larger, somewhat lobed pair, coarsely granular, and mostly paracetabular. There is also a caudal excretory duct, and the main excretory tubes divide anterior to the ventral sucker. Although, this species was con-

sidered to be a member of the Cellulosa subgroup, it has a pair of large oval bodies at the same position in the oral sucker as the virgula organ of the Virgulae subgroup of Xiphidiocercariae; this structure could be a virgula organ!

Gupta and Taneja (1968) described a cercaria from the same snail host, but in a different locality (Pinjore, Panjab, India) and regarded it as "*C. naukuchiensis*" of Malaki and Singh (1962). However, it differs from this latter species in the following respects: anterior pair of the penetration glands larger, coarsely granular, and preacetabular; posterior pair finely granular, smaller, and paracetabular; a different shape of the stylet; the main excretory tubes dividing at the sides of the ventral sucker. Consequently, *C. naukuchiensis* of Gupta and Taneja is not *C. naukuchiensis* of Malaki and Singh, but a different species altogether. All of these authors describe the presence of a caudal excretory duct. As indicated above, how a caudal excretory duct can be present in a xiphidiocercaria, one wonders! In these cercariae there is always a central strand of caudal muscles.

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Sterliadochona pedispicula sp. n. (Nematoda: Spirurinae) from *Salmo gairdnerii* Richardson, and a Discussion of the Genera *Sterliadochona* Skrjabin, 1946 and *Cystidicoloides* Skinker, 1931

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ABSTRACT: The genus *Sterliadochona* Skrjabin, 1946 is removed from synonomy with *Cystidicoloides* Skinker, 1931. The description of *Sterliadochona* is emended and a new species *S. pedispicula* is described. Three new combinations are proposed.

In the literature, the genera *Metabronema* Yorke and Maplestone, 1926, *Cystidicola* Fischer, 1798, *Ichthyobronema* Gnedina and Ssavina, 1930, *Ascarophis* van Beneden, 1871, *Cystidicoloides* Skinker, 1931, and *Sterliadochona* Skrjabin, 1946 have been much confused because nominal species have been misplaced among these groups. In some instances the problem has been made more complex by attempts to stabilize these genera through emendation based upon misidentified species instead of the type-species. Through examination of type-species, Rasheed (1965) established the generic characteristics of *Metabronema*, *Ascarophis*, and *Cystidicola*. Chitwood (1933) negated *Ichthyobronema* by transferring the

type-species *Filaria conoura* Linstow, 1885 to the genus *Rhabdochona*. Rasheed (1965) and Moravec (1967) independently attempted to stabilize the nominal genus *Cystidicoloides*. Moravec made a comparison between the cotypes of *Metabronema truttae* Baylis, 1935 and *Sterliadochona tenuissima* (Zeder, 1800) Spasskiĭ and Roĭtman, 1959 and concluded they were synonymous. Rasheed came to the same conclusion by comparing type material of *M. truttae* with the literature on *S. tenuissima*. Because *M. truttae* had previously been transferred to *Cystidicoloides* by Dollfus and Campana-Rouget, (1956) they independently concluded that the genus *Sterliadochona* was a synonym of *Cystidicoloides*. Both Rasheed and Moravec erred in their attempt to stabilize the genus *Cystidicoloides* by not examining the type-species *C. fischeri* Travassos, Artigas, and Pereira, 1928 and assuming that *M. truttae* was representative of *Cystidicoloides*.

We have examined type specimens of the type-species *C. fischeri* and find the original description to be accurate. Therefore, we believe that both *Cystidicoloides* and *Sterliadochona* are valid genera. The characteristics of *C. fischeri* which distinguish *Cystidicoloides* and *Sterliadochona* are: the development of prominent cuticular extensions on the lateral labia (Fig. 2, A–C), the ratio of the anterior muscular esophagus to the posterior glandular esophagus is greater than 1:10, and the ratio of approximately 1:7 between the distance from the first preanal papillae to the cloacal opening and the length of the long spicule. Both these ratios are less than 1:4 in *Sterliadochona*. These same characteristics place the genus *Cystidicoloides* very close to the genus *Ascarophis*.

The following disposition is proposed for the remaining nominal species previously in *Cystidicoloides*: *C. tenuissima*, *C. harwoodi*, and *C. prevosti* are transferred to the genus *Sterliadochona*. At present *Cystidicoloides* contains only the type-species *C. fischeri*. In addition, *Ascarophis ochracea* (Linstow, 1894) Chitwood, 1933 (recognized as a synonym of *C. tenuissima* by Moravec) is transferred as a valid species to *Sterliadochona*. *Cystidicoloides wardlei* (Smedley, 1934) Rasheed, 1965 not having characteristics of either *Cystidicoloides* or *Sterliadochona* is returned to *Metabronema* as *M. wardlei* Smedley, 1934 *incertae sedis*.

Sterliadochona Skrjabin, 1946

GENERIC DIAGNOSIS EMENDED: Cuticle with distinct transverse striations, posterior margins, in lateral view, appear dentate. The anterior extremity bears subapically four dorsolateral and ventrolateral cephalic papillae, with amphids opening on well developed lateral labia. Stoma two-part, teeth absent, anterior laterally compressed, posterior cylindrical. Esophageal ratio: anterior muscular portion to posterior glandular portion approximately 1:2 or 3. Cervical papillae well developed. Nerve ring encircling muscular portion of esophagus, excretory pore opens just posterior to nerve ring. Female tail conical to obtuse. Vulva near midbody or slightly posterior. Male tail with caudal alae. Posterior ventral portion of male tail with ventral longitudinal ridges. Caudal alae with four pairs of precloacal papillae and six pairs of postcloacal papillae, spicules unequal and dissimilar, gubernaculum absent. Female reproductive system didelphic, amphidelphic. Eggs without polar filaments.

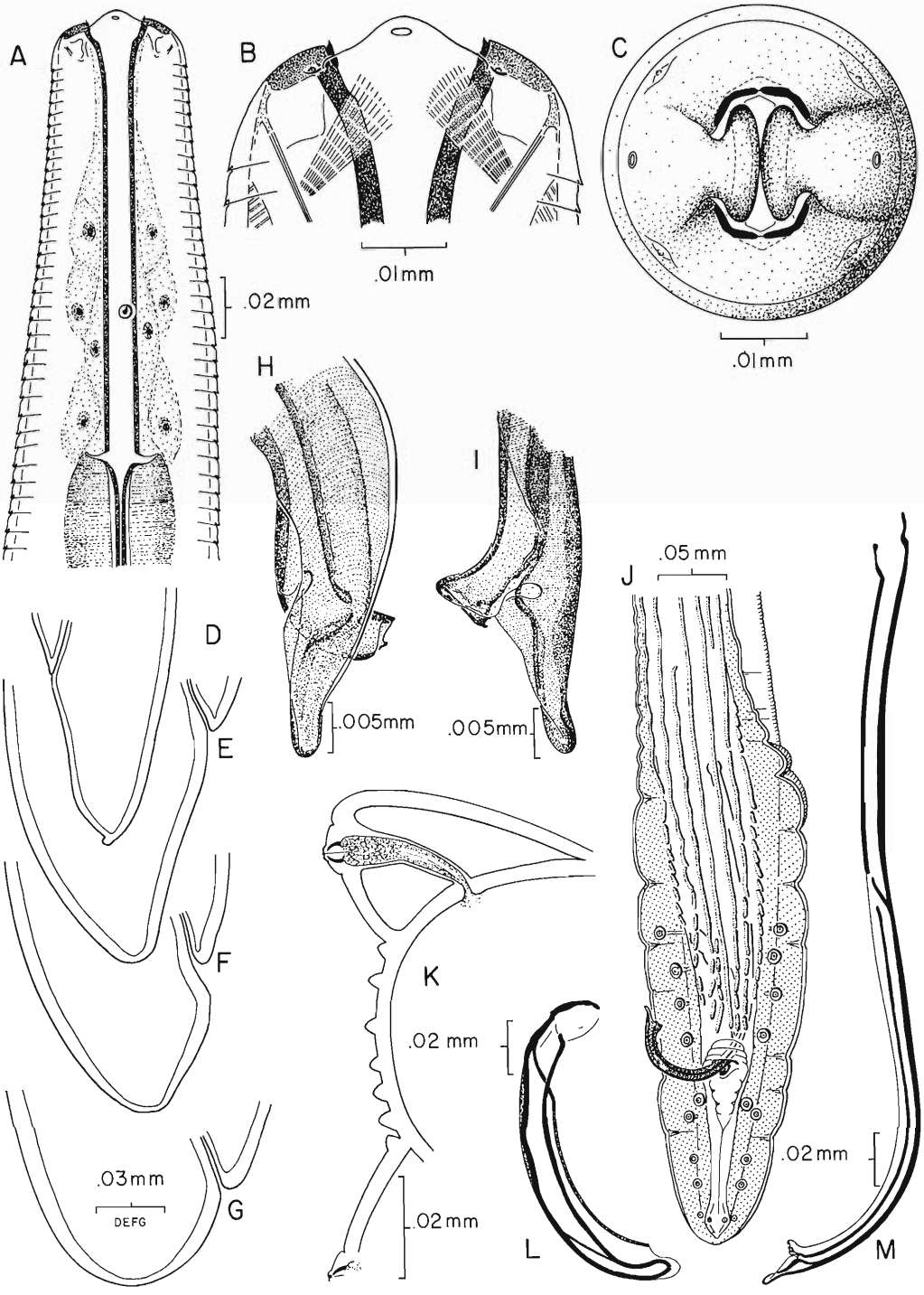
TYPE-SPECIES: *Sterliadochona tenuissima* (Zeder, 1800) Spasskiĭ and Roĭtman, 1959.

SYNONYMS: *Cystidicoloides ssavini* (Skrjabin, 1946) Moravec, 1967; *Cystidicoloides tenuissima* (Zeder, 1800) Rasheed, 1965; *Cystidicoloides canadense* (Skinker, 1931) Rasheed, 1965; *Ichthyobronema ssavini* (Skrjabin, 1946) Spasskiĭ and Roĭtman, 1959; *Cystidicoloides truttae* (Baylis, 1935) Dollfus and Campana-Rouget, 1956; *Cystidicoloides salvelini* (Fujita, 1922) Dollfus and Campana-Rouget, 1956; *Sterliadochona ssavini* Skrjabin, 1946; *Metabronema truttae* Baylis, 1935; *Metabronema salvelini* (Fujita, 1922) Baylis, 1935; *Metabronema canadense* Skinker, 1931; *Ichthyobronema tenuissima* (Zeder, 1800) Gnedina and Savina, 1930; *Cystidicola salvelini* (Fujita, 1922) Fujita, 1928; *Spiroptera salvelini* Fujita, 1922; *Spiroptera tenuissima* (Zeder, 1800) Linstow, 1909; *Ascaris tenuissima* (Zeder, 1800) Rudolphi, 1809; *Fusaria tenuissima* Zeder, 1800.

OTHER SPECIES:

Sterliadochona harwoodi (Chandler, 1931) n. comb.

SYNONYMS: *Ascarophis harwoodi* (Chandler, 1931) Chitwood, 1950; *Metabronema harwoodi* (Chandler, 1931) Baylis, 1934; *Cystidicoloides*



harwoodi (Chandler, 1931) Skinker, 1931; *Cystidicola harwoodi* Chandler, 1931.

Sterliadochona prevosti (Choquette, 1951) n. comb.

SYNONYMS: *Cystidicoloides prevosti* (Choquette, 1951) Dollfus and Campana-Rouget, 1956; *Metabronema prevosti* Choquette, 1951.

Sterliadochona ochracea n. comb.

SYNONYMS: *Cystidicoloides ochracea* (Linstow, 1894) Moravec, 1967; *Ascarophis ochracea* (Linstow, 1894) Chitwood, 1933; *Ichthyobronema ochracea* (Linstow, 1894) Gnedina and Savina, 1930; *Spiroptera ochracea* (Linstow, 1894) Linstow, 1909; *Filaria ochracea* Linstow, 1894.

Sterliadochona pedispicula sp. n. (Figure 1, A–M)

DIMENSIONS: *Females*—L = 9.75–17.6 mm, a = 71–107, b = 2.8–4.1, c = 175–440, V = 55–61, stoma = 0.122–0.150 mm, anterior esophagus = 0.85–1.41 mm, posterior esophagus = 2.3–3.2 mm, total esophagus = 3.1–4.6 mm, cervical papillae = 0.097–0.119 mm, excretory pore = 0.19–0.27 mm, nerve ring = 0.165–0.200 mm, eggs = 40–50 μ × 20–30 μ.

Males—L = 5.79–8.7 mm, a = 64–108, b = 2.1–2.7, c = 45–87, stoma = 0.103–0.141 mm, anterior esophagus = 0.68–0.95 mm, posterior esophagus = 1.54–2.31 mm, total esophagus = 2.2–3.24 mm, cervical papillae = 0.075–0.124 mm, excretory pore = 0.222–0.256 mm, nerve ring = 0.145–0.172 mm, left spicule = 0.312–0.353 mm, right spicule = 0.119–0.141 mm.

MALE (HOLOTYPE): L = 6.66 mm, a = 74, b = 2.5, c = 63.4. Body slender, tapering more anteriorly than posteriorly. Greatest body width 0.09 mm. Cuticle with pronounced transverse striations, posterior margins slightly overlap anterior margin of next annule. Annulation at stoma base 4.6 μ wide. Lateral lips, bearing amphids, well developed. Subdorsal and subventral labia not developed, subdorsal and subventral sectors demarcated by cheilorhabdions, tooth-like in lateral view. Stoma 0.116 mm long, right cervical papilla 0.121 mm from anterior extremity, left cervical papilla 0.107 mm from anterior end. Total esophageal length 2.7 mm, anterior portion 0.87 mm long (measured from anterior extremity), posterior portion 1.83 mm long. Nerve ring encircles anterior portion of muscular esophagus 0.169 mm from anterior end. Excretory pore 0.249 mm from anterior extremity. Subventral caudal alae present approximately 0.430 mm long. Alae with four precloacal and six postcloacal pairs of papillae. The most caudal pair not pedunculate. Most anterior pair of precloacal papillae 0.107 mm from cloacal opening. Posteriorly male tail with ventral longitudinal ridges, seven at the level of the first pair of precloacal papillae. Spicules unequal and dissimilar, left spicule 0.315 mm long, right spicule 0.135 mm long. Left spicule distally elaborated into foot-like process. Tail length 0.105 mm.

FEMALE (ALLOTYPE): L = 12.62 mm, a = 108, b = 3.3, c = 324, V = 59. Head structures and cuticle similar to male. Transverse striae 4.6 μ apart at base of stoma. Stoma 0.131 mm long, right cervical papilla 0.111 mm, left cervical papilla 0.095 mm from anterior extremity. Total length of esophagus 3.82 mm, anterior portion 1.12 mm (measured from anterior extremity) posterior portion 2.70 mm. Nerve ring circles anterior portion of muscular esophagus 0.180 mm from anterior end. Excretory pore 0.245 mm from anterior. Vulva raised, located 7.46 mm (59%) from anterior end, guarded by anterior flap 8 μ long. *Vagina vera* 0.076 mm, vagina uterina posteriorly directed 0.189 mm. Eggs 35.3–40 μ × 22–24 μ, shell thickness 2.6 μ. Tail 0.039 mm, rounded.

HOLOTYPE: Male, collected October 3, 1969 by A. R. Maggenti, catalogue No. 182 UCNC, Davis.

PARATYPES: Two males, Nos. 183–184 UCNC, Davis; two females, Nos. 185–186 UCNC, Davis. One male and one female, Para-

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Figure 1. ***Sterliadochona pedispicula.*** **A, Female, head; B, Female head, showing cheilorhabdions in lateral view; C, Female, *en face*, figuring lateral lips and cheilorhabdions; D, Larval tail; E–G, Female, tail tip variation; H, distal tip, left spicule, dorsal view; I, distal tip, left spicule, lateral view; J, ventral view male tail; K, Cross-section male tail, at level of first precloacal papillae; L–M, toto-view, right and left spicule, respectively.**

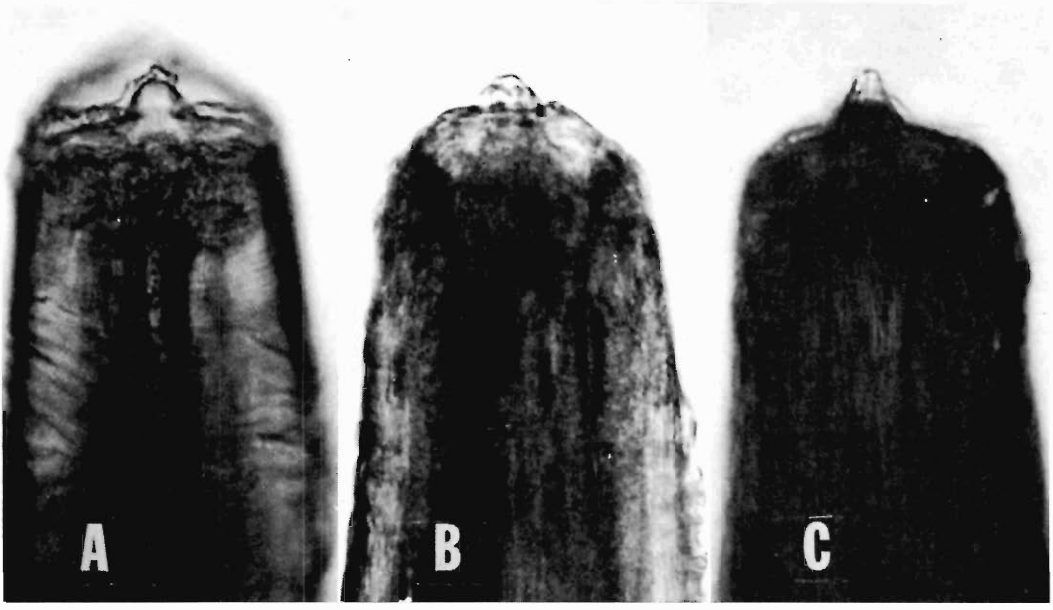

Figure 2. ***Cystidicola fischeri.*** **Photographs showing heads of type material. A, B, female; C, male.**

sitological Laboratory, USDA, Beltsville, Maryland.

TYPE HOST: *Salmo gairdnerii.* (Rainbow trout.)

HABITAT: Stomach and esophagus.

TYPE LOCALITY: Jawbone Creek, T. IN, R. 18E, Stanislaus National Forest, Tuolumne County, California.

DIAGNOSIS: *Sterliadochona pedispicula* can be distinguished from other members of the genus by the distal end of the right spicule and by the unusual foot-like development of the distal end of the left spicule. Both males and females can be distinguished from species other than *S. ochracea* by the pronounced development of the cheilorhabdion plates. It can be distinguished from *S. ochracea* by the greater stomatal length.

Acknowledgments

The authors wish to thank Dr. J. F. Teixeira de Freitas of the Institute of Oswaldo Cruz for the loan of type specimens (No. 6077) of *Cystidicola fischeri* and Mr. W. W. Becklund of the USDA Beltsville Parasitological Laboratory for the loan of type material of *Metabronema canadense* (USNM Helm. Coll. 8035) and *Cystidicola harwoodi* (USNM Helm. Coll. 8099).

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Intestinal Parasites and Commensals of an Indigenous Population in the Lake Baringo Area of Central Kenya[1]

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ABSTRACT: Examinations of a stool sample from each of 215 natives of the Lake Baringo area of central Kenya revealed the presence of common protozoa and helminths in the population. Twenty-five per cent of samples contained *Entamoeba histolytica*; 12%, *E. hartmanni*, and 54, 34, and 31%, respectively, contained *E. coli*, *Endolimax nana*, and *Iodamoeba bütschlii*. Cestodes were represented by *Hymenolepis nana* (2%) and *Taenia* (9%), the nematodes by *Enterobius vermicularis* (1%), hookworm (4%), *Strongyloides* (1%), *Trichostrongylus* (8%), and *Trichuris trichiura* (5%). *Ascaris* was absent. Three percent of samples contained eggs of *Fasciola hepatica*.

Little is known of the parasite diseases in some populations of Africa in spite of the fact that many populations have some contact or degree of association with survey type studies or with hospital services. The present report is based upon a study of fecal samples obtained from a human population in the central part of the Rift Valley Province, Kenya, where epidemiological factors may drastically affect parasite infections. Even though cursory in nature, data obtained indicate the relative prevalence of intestinal commensals and parasites.

Materials and Methods

Over a period of three weeks in February 1968, a single fecal specimen was obtained from each of 215 natives who came to the Marigot area as outpatients or members of families to be examined and treated at the Marigot Health Center. Marigot, a small village a few miles southwest of Lake Baringo, is located in a desert-grass bush or dry bush, scattered-trees habitat, with an annual rainfall of 20 to 30 inches. This area, at 3,000 ± feet, lies in the ethnographic area classed as Nilo-Hamatic. The Lake Baringo area is a mixing point, with the presence and overlap of several indigenous elements, including Mjems, Suks (Pokots), Tukens, Luos, and Kipsigis. Although some individuals in this study were interrogated to obtain general epidemiological information, no attempt has been made to segregate data by ethnic or locality groups.

Containers for feces were handed to contributors several hours before collection of materials. Fecal samples were fixed at the health center as soon as feasible after passage. One gram, consisting of several samples selected at random from the entire stool, was fixed in 10% formalin in 15 ml capacity vials. Samples were examined in the laboratory at the Southwest Foundation for Research and Education (SFRE) after return to the United States. First, a direct smear, consisting of several drops, was drawn by eyedropper pipette from the undisturbed upper layer of sediment in each vial. Approximately half of the remaining sample was subjected to the formalin–ether concentration technic of Ritchie (1948). The prevalence of commensals and parasites (Table 1) is indicated for the direct and the concentration technics. Also, there is an indication of prevalence as determined by use of both technics.

Findings and Discussion

Parasite infections in indigenous peoples is taken more or less for granted and accepted as a consequence of living in habitats which foster parasite transmission. Attention, as a rule, is given to parasitoses only when unusual disease situations arise, or when a population becomes noticeably affected by such debilitating parasite diseases as malaria, ancylostomiasis, filariasis or schistosomiasis. The present report, in the absence of pertinent epidemiological information, is concerned primarily with the occurrence of intestinal fauna in persons examined at a given time.

Although the Kenya government has spon-

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Table 1. Prevalence (%)* of intestinal parasites and commensals in peoples of Marigot, Rift Valley Province, Kenya.

Parasites and Commensals	Vial Sed. Exam.	Formalin–Ether Conc.	Combined Exams.
PROTOZOA			
AMOEBAE			
Entamoeba histolytica	19	21	25
Entamoeba hartmanni	6	11	12
Both *E. histolytica* and *E. hartmanni*	1	3	3
Entamoeba coli	40	53	54
Endolimax nana	26	32	34
Iodamoeba bütschlii	24	28	31
FLAGELLATES			
Chilomastix mesnili	4	4	5
Giardia lamblia	6	6	9
HELMINTHS			
CESTODES			
Hymenolepis nana	1	2	2
Taenia (saginata?)	5	8	9
NEMATODES			
Enterobius vermicularis	2	4	4
Hookworm	2	4	4
Strongyloides stercoralis	0	1	1
Trichostrongylus sp.	2	6	8
Trichuris trichiura	1	4	5
TREMATODES			
Fasciola hepatica	1	2	3
Stools without protozoa or helminths	25	19	16
Number of stools examined	215	215	215

* Value given as nearest whole number.

sored health programs and provided varied medical help to different populations in the bush as well as urban centers, reports, except for hospital records, on the incidence of intestinal parasites and commensals are limited. Briscoe (1929) has given the incidence of helminths in patients admitted to a hospital in the Kitui District, Roberts (1949a, b) has provided information on the occurrence of protozoa and helminths in several hundred African school children, and Philip (1927) noted the helminths found in peoples from the coastal area of Kenya. More recently, Moore and Roberts (1958) have given the incidence of some of the more important parasites recorded in a medical survey in the Kisii District of Kenya, and Wagner and Hitman (1963) have reported on the prevalence of intestinal protozoa and helminths in children in a mission hospital. It is difficult to assess, and impractical to compare, the parasitological conditions in different communities since there are great divergences in ethnic customs and epidemiological factors are variable. However, generalizations may be made for parasitism evident in the people of certain areas. Thus, Heisch (1947), in reporting on medical work in the Northern Frontier District of Kenya, stated that tapeworm was present, but helminth infections, in general, appeared to be rare.

Even though the incidence of *E. histolytica* varies markedly from one population to another, the occurrence of this parasite in a quarter of persons examined is not surprising with an incidence of 54% for *E. coli*, 34% for *Endolimax nana*, and 31% for *Iodamoeba bütschlii*. Our figure for *E. histolytica* is much higher than that found by Cherop (8%) (personal communication) in people examined in the same clinic the previous year, as well as that given by Wagner and Hitman (1963) in a recent survey of school children.

With few exceptions, the prevalence of helminths satisfied expectations for the population under study. The most noteworthy record is the absence of *Ascaris lumbricoides* which occurs in many populations of the world and commonly in other surveys in Kenya (Briscoe, 1929; Moore and Roberts, 1958). This is the first negative record in a number of surveys conducted by Kuntz and coworkers (Kuntz et al., 1955, 1958) in other parts of Africa. Cherop (personal communication) recorded *Ascaris* eggs in 13% of stools processed in the Marigot clinic earlier, but Wagner and Hitman (1963) indicated the presence of *Ascaris* in only 3.9% of Kenya children examined. The figure of 9% for *Taenia* infection is considerably lower than that found in other Kenya populations sampled by Briscoe (1929), Froyd (1965), Roberts (1949a), and by Cherop (45%) earlier in Marigot. *Fasciola hepatica* is found only infrequently in man, but it is not entirely unexpected in indigenous peoples who associate closely with cattle, goats, and wild herbivores which provide a means of infection for lymnaead snails around water sources shared by man. There is no way to judge whether these are genuine or spurious infections.

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Nematode Parasites of Oceanica. XV. Acuariidae, Streptocaridae, and Seuratidae of Birds[1]

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ABSTRACT: *Acuaria kinsellai* sp. n. is described from the black drongo, *Dicrurus macrocercus harterti*, from Taiwan. It is nearest to *A. spinosa*, differing in the length of cordons of the male, which are 6.9 mm long, and in having spicules 700 and 300 μ long. *Rusguniella microcordonis* sp. n. is described from the ruddy kingfisher, *Halcyon coromanda major*, from Taiwan. It is differentiated from other species in the genus by its extremely small cordons and by possessing 13 or 14 pairs of preanal papillae. Also reported and briefly discussed are *Acuaria chordata* (Mueller, 1897) Gendre, 1920; *Paracuaria somateriae* (Ryjikov, 1960) Leonov, Zimbaluk et Belgurov, 1963; *Synhimantus laticeps* (Rudolphi, 1819) Railliet, Henry et Sisoff, 1912; *Desportesius spinulatus* Chabaud et Campana, 1949; *Dispharynx nasuta* (Rudolphi, 1819) Railliet, Henry et Sisoff, 1912; and *Skrjabinura spiralis* Gnedina, 1933.

The specimens on which this report is based were collected by the second author and his associates of Naval Medical Research Unit No. 2 on expeditions to Sabah, Malaysia, and Palawan, Republic of the Philippines, as well as during field operations in Taiwan. The worms were killed in hot alcohol and stored in 70% alcohol and glycerine. Clearing for study was by dehydration in glycerine. Host names were taken from Kuntz (1969a, 1969b), and Kuntz and Dien (1970). All measurements are in microns unless otherwise indicated.

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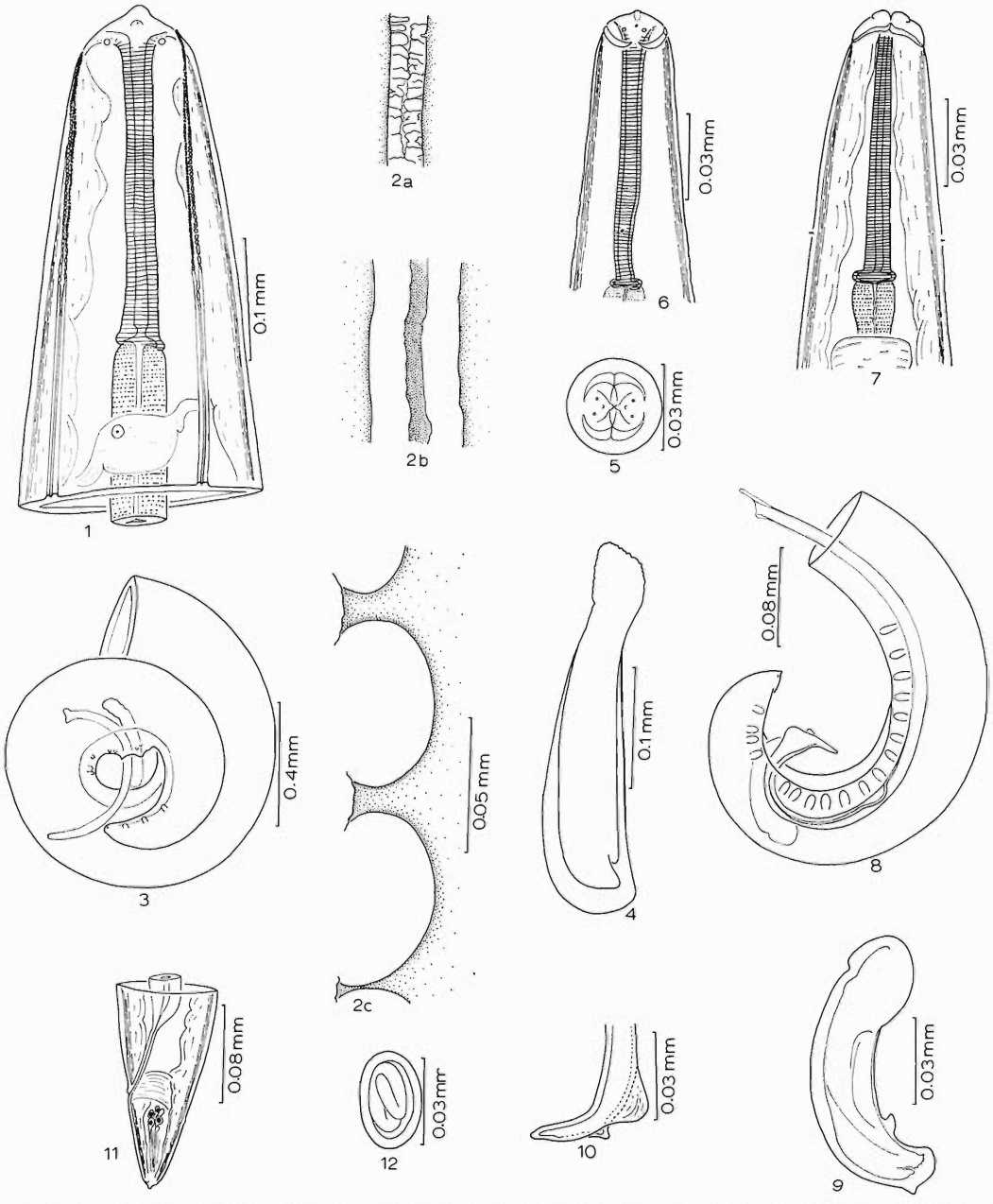

Figures 1–4. ***Acuaria kinsellai*** **sp. n. from black drongos in Taiwan. 1. Anterior end of holotype male, lateral view. 2a, b, c. Cordons, near anterior end, middle, and posterior end, all at same scale as 2c. 3. Tail of male, lateral view. 4. Right spicule, lateral view.**

Figures 5–12. ***Rusguniella microcordonis*** **sp. n. from kingfishers in Taiwan. 5. En face. 6. Head end, lateral view. 7. Head end, dorsal view. 8. Tail of male, lateral view. 9. Right spicule, lateral view. 10. Tip of left spicule, lateral view. 11. Tail of female, lateral view. 12. Egg.**

Acuariidae Seurat, 1913

The following is a report on the Acuariinae Railliet, Henry et Sisoff, 1912, in our collection. The Echinuriinae Sobolev, 1943, were previously reported (Schmidt and Kuntz, in press).

Acuaria kinsellai sp. n. (Figs. 1–4)

One male and two females were found under the gizzard linings of three black drongos, *Dicrurus macrocercus harterti* Baker, 1918, in Taiwan. They differ so markedly from all known nematodes that we consider them to represent a new species, named in honor of Dr. John M. Kinsella.

DESCRIPTION: Cuticle with large cross-striations. Anterior end (Fig. 1) with two prominent lateral pseudolabia, each tipped with a large, rounded cuticular tooth. Two cephalic papillae and prominent amphid near base of each pseudolabium. Cordons (Figs. 2a, b, c) spinous at anterior end, becoming smooth, shallow grooves, then becoming a series of large longitudinal ridges. Pharynx long, with cross-striations. Nerve ring posterior to junction of pharynx and muscular esophagus. Muscular esophagus slender, glandular esophagus very long. Junction of muscular with glandular esophagus conspicuous.

MALE: 14.5 mm long, 345 greatest width. Cordons 6.9 mm long. Nerve ring 360, excretory pore 585, deirids 360 from anterior end. Pharynx 260, muscular esophagus 840, glandular esophagus 3.68 mm long. Tail (Fig. 3) 600 long, tightly coiled. Caudal alae slender. Caudal papillae asymmetrically arranged as follows: preanal-5 on left side, 3 on right side; 2 pairs adanal; postanal-3 on left side, 4 on right side. Phasmidial pores near tip of tail. Left spicule 700 long, with notch in tip; right spicule (Fig. 4) 300 long, stout, with simple, rounded tip. Both spicules with irregular transverse markings; right spicule with thick, transparent cortex on distal half, not to be confused with spicule sheath.

FEMALE: 25.0–35.0 mm long, 400 to 535 greatest width. Cordons 7.4–9.66 mm long. Nerve ring 335–425, excretory pore 560–630, deirids 370–400 from anterior end. Pharynx 240–295, muscular esophagus 0.690–1.15 mm, glandular esophagus 4.0–6.2 mm long. Tail 375–480 long. Vulva 9.3–11.0 mm from anterior end. Eggs 46–48 by 32.

TYPE HOST: Black drongo, *Dicrurus macrocercus harterti* Baker, 1918, (Passeriformes: Dicruridae).

LOCATION: Under koilon of gizzard.

TYPE LOCALITY: Hua-lien, Hua-lien Hsien, Taiwan. Also collected near Sun Moon Lake, Nan-tou Hsien, Taiwan.

TYPE SPECIMENS: USNM Helm. Coll. holotype male no. 71968, allotype female no. 71969, paratype female no. 71970.

REMARKS: In body size and in shapes and approximate sizes of the spicules, *A. kinsellai* is nearest to *A. spinosa* Cram, 1927, from gallinaceous birds from North America. Further, *A. spinosa* is one of the four species in the genus reported to have spinous cordons like the present species. *A. kinsellai* differs from *A. spinosa* in the following ways: (1) cordons of male *A. kinsellai* are about 6.9 mm long, compared with 0.495 mm in *A. spinosa*; (2) the spicules of *A. kinsellai* are 700 and 300 long, a ratio of 2.3:1, while they are 700 to 720 and 192 long in *A. spinosa*, a ratio of about 3.7:1.

The other species reported to have spinous cordons are *A. centrocerci* Simon, 1939; *A. multispinosa* Pérez Vigueras, 1937; and *A. cordonspinosa* Barus et Garrido, 1968. None of the males of these has cordons over 3.0 mm long, compared with 6.9 mm in *A. kinsellai*. Among the 70 or more species of *Acuaria* there may be species with spinous cordons which escaped the notice of their authors. However, published descriptions of all of these species show none of them to have spicules approximating the sizes and shapes of our specimens. The spicules and extreme lengths of the cordons and glandular esophagus will serve as easy characters for future recognition of *A. kinsellai*.

Acuaria chordata (Mueller, 1897) Gendre, 1920

One male and one female were found in the gizzards of a haircrested drongo, *Dicrurus hottentottus palawanensis* Tweeddale, and an ashy drongo, *D. l. leucophaeus* Vieillot, in Puerto Princessa, Palawan, and a second female was recovered from the gizzard of a black drongo, *D. macrocercus harterti* from Hua-lien, Hua-lien Hsien, Taiwan. The species is well known from a variety of passeriform birds in Europe,

Asia, Africa, and South America, but our report constitutes new host and distribution records.

SPECIMENS DEPOSITED: USNM Helm. Coll. nos. 71811–71813.

Paracuaria somateriae (Ryjikov, 1960) Leonov, Zimbaluk et Belgurov, 1963

Four males and 8 females were found under the koilons of 2 domestic ducks, *Anas platyrhynchus* L., from Pu-yen, Chang-hua Hsien, and a single male was found under the koilon of a white-breasted water hen, *Amaurornis phoenicurus chinensis* Boddaert, at Pu-li, Nan-tou Hsien, Taiwan. It has been previously reported from several species of ducks in Russia. The water hen is a new host record and Taiwan is a new locality record. The cordons of this species are very small and difficult to see, even with an oil immersion, phase contrast lens. For this reason, Chabaud and Petter (1959) consider *Paracuaria* Rao, 1951, to be the most primitive of the Acuariinae.

SPECIMENS DEPOSITED: USNM Helm. Coll. nos. 71817–71819.

Synhimantus laticeps (Rudolphi, 1819) Railliet, Henry et Sisoff, 1912

Two females were found in the proventriculus of a kestrel, *Falco tinnunculus intersticus* Horsfield, at Wu-lai, Tai-pei Hsien, Taiwan. This well-known species has been reported from a variety of hawks and owls, including the kestrel, In Europe, India, Russia and Africa. This is the first record from Taiwan.

SPECIMENS DEPOSITED: USNM Helm. Coll. no. 71833.

Desportesius spinulatus Chabaud et Campana, 1949

Numerous specimens were obtained from 3 little egrets, *Egretta g. garzetta* L., from Tao-youn, Tao-youn Hsien, and Tai-pei, Tai-pei Hsien, Taiwan; 6 cattle egrets, *Bubulcus ibis coromandus* (Boddaert); and a lesser egret, *Egretta intermedia pelleuca* Deignan, from Chi-hu, Chang-hua Hsien, Shin-she, Tai-chung Hsien, and I-lan, I-lan Hsien, Taiwan. It is a common parasite of Ardeiformes in Europe, Asia, and Africa, but has not previously been reported from Taiwan. The parasite reported as *Cosmocephalus* by Ryjikov and Hohlova in Skrjabin, Sobolev, and Ivashkin (1965) is apparently this species.

SPECIMENS DEPOSITED: USNM Helm. Coll. nos. 71839–71847.

Dispharynx nasuta (Rudolphi, 1819) Railliet, Henry et Sisoff, 1912

This parasite was recovered from the following hosts and localities: kite, *Milvus l. lineatus* Gray, Hua-lien, Hua-lien Hsien; vinous-throated parrotbill, *Paradoxornis webbianus bulomachus* (Swinhoe), Hua-lien, Hua-lien Hsien; lesser coucal, *Centropus toulou takatsukasai* Momiyama, Shin-chu, Shin-chu Hsien; dusky thrush, *Turdus naumanni eunomus* Temminck, Hua-lien, Hua-lien Hsien; blue rock thrush, *Monticola solitarius philippensis* (P.L.S. Muller), Ma-kung, Peng-hu Hsien, Taiwan. All are new host records and Taiwan is a new locality for this cosmopolitan parasite of birds.

SPECIMENS DEPOSITED: USNM Helm. Coll. nos. 71834–71838.

Streptocaridae Skrjabin, 1941

Rusguniella microcordonis sp. n. (Figs. 5–12)

Numerous specimens were found under the koilons of the gizzards of two ruddy kingfishers, *Halcyon coromanda major*, in Taiwan. The following description is based on these specimens.

DESCRIPTION: Body slender, delicate. Anterior end (Figs. 5, 6, 7) with two lateral pseudolabia, each tipped with a cuticular tooth. Two cephalic papillae and an inconspicuous amphid present near base of each lip. Buccal capsule dorsoventrally elongated, not sharply delineated from pharynx. Pharynx long, with conspicuous cross-striations. Nerve ring posterior to junction of pharynx and esophagus. Muscular esophagus slender, glandular esophagus long. Deirids inconspicuous, simple.

MALE (10 mature specimens): 7.0–8.5 mm long, 90–108 greatest width near middle of body. Pharynx 90–106 long. Excretory pore 72–86, nerve ring 110–122, deirids 70–90, from anterior end. Muscular esophagus 400–465, glandular esophagus 890–960 long. Tail (Fig. 8) bluntly pointed, 70–95 long. Right spicule (Fig. 9) stout, with blunt tip and ventral, sub-

apical tooth; 85–100 long. Left spicule with complex tip (Fig. 10) bearing conspicuous flange; 370–440 long. Caudal papillae as follows: 4 pairs postanal, 13 or 14 pairs preanal. Phasmidial pores near tip of tail.

FEMALE (10 gravid specimens): 10.5–12.5 mm long, 130–150 greatest width at about middle of body. Pharynx 95–110 long. Excretory pore 70–90, nerve ring 110–130, deirids 65–90, from anterior end. Muscular esophagus 400–500, glandular esophagus 800–960 long. Tail (Fig. 11) bluntly pointed, 80–110 long. Vulva about equatorial, 5.5–6.1 mm from anterior end. Eggs (Fig. 12) oval, embryonated when laid, 34–38 long by 22–24 wide.

TYPE HOST: ruddy kingfisher, *Halcyon coromanda major* Temminck et Schlegel, 1848. (Coraciiformes: Alcedinidae).

LOCATION: Under koilon of gizzard.

LOCALITIES: Wu-lai, Tai-pei Hsien (type locality); Kuan-yin-shan, Tai-pei Hsien; Taiwan.

TYPE SPECIMENS: USNM Helm. Coll. holotype male no. 71971, allotype female no. 71972, paratypes nos. 71973, 71974.

REMARKS: *Rusguniella microcordonis* is easily differentiated from other species in the genus by the small size of its cordons and the large number of preanal papillae. The cordons are very delicate and visible only with a high resolution, oil-immersion lens. For this reason it is possible that *Viktorocara tenuis* (Maplestone, 1932) Skrjabin, Sobolev et Ivashkin, 1965, may be the same species. The tails of the males are strikingly similar. However, neither Maplestone (1932) nor Singh (1949) in their descriptions of the species mention the presence of cordons or describe or illustrate the complex tip of the left spicule. Similarly, *Schistogendra incisa* Chabaud et Rousselot, 1956, is remarkably similar to the species at hand, differing mainly in that the inner margins of the pseudolabia are deeply scalloped. Small cuticular plaques are illustrated on the head of the species which are similar to the cordons of *R. microcordonis*. Careful reexamination of the type specimens of this species may show it be conspecific with *R. microcordonis*. Until then we have no recourse but to consider our specimens as representing a new species.

Rusguniella alcedonis Yamaguti et Mitunaga, 1943, from the common kingfisher, *Alcedo atthis* Gmelin, is the only species in the genus reported to date from Taiwan. It is easily differentiated from *R. microcordonis* by having massive cordons and only four or five pairs of preanal papillae. It should be pointed out that on the basis of published descriptions *Alcedospirura collaricephala* Oschmarin, 1959, cannot be distinguished from *R. alcedonis*. Skrjabin, Sobolev, and Ivashkin (1965) place *A. collaricephala* in *Aviculariella* Wehr, 1931, a genus which Jõgis (1963) and the present authors consider synonymous with *Rusguniella*. Therefore, we consider *A. collaricephala* to be a junior synonym of *R. alcedonis*.

Rusguniella skrjabini Chuan, 1961

Two females attributed to this species were found in the gizzard of a wood sandpiper, *Tringa glareola* L., from Ranau, Sabah, Borneo, representing new host and locality records. The species is known from *Tringa* spp. in Russia.

SPECIMENS DEPOSITED: USNM Helm. Coll. no. 71816.

Seuratidae Hall, 1916

Skrjabinura spiralis Gnedina, 1933

(Syn.: *Seuratinema brevicaudatum* Johnston et Mawson, 1941; *Seuratinema pomatostomi* Johnston et Mawson, 1941; *Seuratinema magnum* Johnston et Mawson, 1941; *Skrjabinema brevicaudatum* (Johnston et Mawson, 1941) Mawson, 1960; *Skrjabinema magnum* (Johnston et Mawson, 1941) Mawson, 1960; *Skrjabinura smurociuris* Sobolev, 1960; *Skrjabinura petterae* Vassiliades, 1970).

This species was first reported from Russia by Gnedina (1933) who found it in a nighthawk, *Caprimulgus europaeus*. It has not been reported again under the original name. Johnston and Mawson (1941a) established *Seuratinema* as a new genus from an Australian hawk, stating a gubernaculum was absent, and also (1941b) added two more species to the genus. Later, Mawson (1960) synonymized the two genera, after reexamining the specimens described as *Seuratinema*, but accidentally used the name *Skrjabinema* Gnedina, 1933, rather than *Skrjabinura* Gnedina, 1933. This was corrected later in the same volume. (*Skrjabinema* Wereschtchagin, 1926, is an oxyurid found in ruminants). We are unable to distinguish the three Australian species from *S. spiralis*, from the published descriptions.

Sobolev (1960) described *Skrjabinura smur-*

ociuris from the little owl, *Athene noctua*, in Russia. It is differentiated from *S. spiralis* mainly on spicule length, 280 microns compared to 320 to 340 in *S. spiralis*. Vassiliades (1970) described *Skrjabinura petterae* from cuculiform, caprimulgiform and passeriform birds in Madagascar. He was apparently unaware of the paper of Sobolev (1960), and compared his specimens only with *S. spiralis* and the three Australian species, from which they differ in possessing minute "denticles" on the anterior end of the esophagus, and in the size of the spicules, 280 μ. His measurements overlap those of *S. smurociurus*.

We have obtained numerous specimens of *Skrjabinura* from the following localities and hosts. Sabah: malcoha, *Phaenicophaeus c. chlorophaeus* (Raffles); chestnut-breasted malcoha, *P. curvirostris microrhinus* Berlepsch (Cuculiformes); green magpie, *Kitta chinensis minor* (Cabanis) (Passeriformes). Palawan: white-collared kingfisher, *Halcyon chloris collaris* (Scopoli) (Coraciiformes); lesser coucal, *Centropus bengalensis javanensis* (Dumont) (Cuculiformes). Taiwan: brown hawk owl, *Ninox scutulata japonica* (Temminck et Schlegel) (Strigiformes); bamboo partridge, *Bambusicola thoracica sonorivox* Gould (Galliformes); lesser coucal, *Centropus toulon takatsukasai* Momiyama (Cuculiformes).

Studies on these specimens indicate considerable intraspecific variation, encompassing the ranges of all species described in the genus. Spicule lengths ranged from 280–340 μ, and gubernaculum lengths from 300–375 μ, such differences occurring in worms from the same bird. Minute denticles on the anterior end of the esophagus can be seen in some specimens but not all. It is possible that they occurr in those collected by other authors but have not previously been noticed. This would suggest that there is only one species so far discovered, *S. spiralis*, and that it is somewhat variable in morphology and exhibits little host specificity.

SPECIMENS DEPOSITED: USNM Helm. Coll. nos. 71542–71550.

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Eimeria paynei sp. n. (Protozoa: Eimeriidae) from the Gopher Tortoise, *Gopherus polyphemus*[1]

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ABSTRACT: *Eimeria paynei* sp. n. is described from the gopher tortoise, *Gopherus polyphemus*, in Georgia. The ellipsoidal sporulated oocysts of *E. paynei* are 19–26 μ by 16–20 μ (mean, 23.2 by 18.6 μ). An oocyst residuum is absent and a polar granule is present. The ovoid sporocysts are 12–14 μ by 7–9 μ (mean, 13.2 by 8.1 μ). The sporocyst residuum is a mass of many small granules enclosed by a thin membrane. This is the first description of an eimerian oocyst from *Gopherus polyphemus*.

In elucidating the possible ecological complexities and particularly the paratenic hosts of the spirurids of swine, examinations of wildlife are being carried out at the Animal Parasite Research Laboratory in Tifton, Georgia. During a parasite survey of turtles from south Georgia, a large number of coccidian oocysts, herein described as a new species, was found in the feces of a gopher tortoise, *Gopherus polyphemus*, from Tift County.

Feces from the tortoise were sporulated in 2.5% $K_2Cr_2O_7$ at room temperature for 3 weeks, concentrated with Sheather's solution, and examined microscopically at 1,000× with a planapochromatic objective. The size range and mean (in parentheses) of 100 oocysts and sporocysts were determined with an ocular micrometer. All measurements are in microns.

Eimeria paynei sp. n.

Oocysts (Fig. 1) ellipsoidal. Oocyst wall 2 layers (proven by separating the 2 layers by sliding the coverslip back and forth over the oocyst): the outer layer lightly pitted, brownish-yellow in color, and about 0.5 thick on the sides, thinning to about 0.25 on the ends; inner layer colorless to light brown, and about 1 thick. Micropyle absent. Sporulated oocysts 19–26 by 16–20 (23.2 by 18.6); length–width ratios 1.1 to 1.4 (1.25). Oocyst residuum absent. One to 3 ellipsoidal or subspherical polar granules present; other smaller granules, probably polar granule fragments, often present. Sporocysts ovoid, 12–14 by 7–9 (13.2 by 8.1), with a Stieda body at the pointed end. Spheroidal or ellipsoidal sporocyst residuum composed of many small homogeneous granules enclosed by a thin membrane. Sporozoites elongate, lying lengthwise in the sporocysts, partially curled around each other. Single large refractile body at the broad end of each sporozoite.

Discussion

Sixteen species of *Eimeria* have been described from turtles. Thirteen of these species were reviewed by Pellérdy (1965). In addition, a new species was described from *Pseudemys ornata* (Lainson, 1968), from *Pseudemys scripta* (Sampson and Ernst, 1969), and from *Chelydra serpentina* (Ernst et al., 1969). Of these sixteen species, only two were from members of the Testudinidae, the land tortoises to which *Gopherus* belongs. Cerruti (1930) described *Eimeria brodeni* from the

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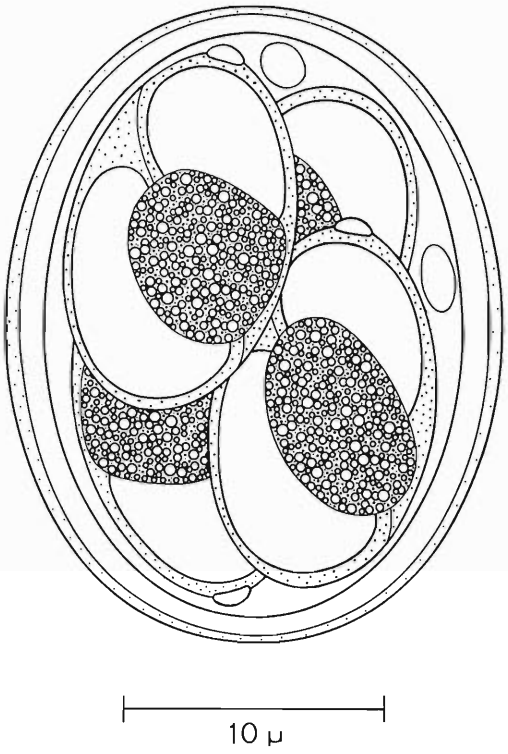

Figure 1. Sporulated oocyst of *Eimeria paynei*.

Greek tortoise, *Testudo graeca*; and Carini (1942) described *Eimeria jaboti* from the South American tortoise, *Testudo tabulata*.

The sporulated oocysts of *Eimeria paynei* differ from the sporulated oocysts of *E. brodeni* by having shorter oocysts and larger sporocysts and by lacking a micropyle. The length of the oocysts of *E. paynei* is 19–26, whereas the length of the oocysts of *E. brodeni* is 28–32. The sporocysts of *E. paynei* are 12–14 by 7–9, and those of *E. brodeni* are 10 by 6–7. A distinct micropyle is present on the oocyst wall of *E. brodeni*.

Eimeria paynei sporulated oocysts differ from *E. jaboti* sporulated oocysts by having a different shape, larger oocysts, and a Stieda body on the sporocysts. *E. paynei* oocysts are ellipsoidal, whereas *E. jaboti* oocysts are subspherical. The oocysts of *E. paynei* are 19–26 by 16–20; those of *E. jaboti* are 17–19 by 15–17. The sporocysts of *E. jaboti* do not have a Stieda body.

Eimeria paynei is named in honor of Dr. Jerry A. Payne, USDA Southeastern Fruit and Nut Tree Research Station, Byron, Georgia. Dr. Payne collected many of the turtles used in our parasite survey.

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Studies on Helminths of North Dakota. II. Parasites of the Badger, *Taxidea taxus* (Schreber)*

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ABSTRACT: Seventeen badgers, *Taxidea taxus* (Schreber), from North Dakota were examined for helminths. The parasites recovered are: *Alaria* (*Paralaria*) *taxideae*, *Euparyphium melis*, *Atriotaenia* (*Ershovia*) *procyonis*, *Monordotaenia taxidiensis*, *Ancylostoma taxideae*, *Ascaris columnaris*, *Filaria taxideae*, *Molineus patens*, and *Physaloptera torquata*. Several of these constitute new distribution records and *E. melis* a new host record. Of 44 additional badgers examined for cestodes only, six were infected with *A. procyonis* and eight with *M. taxidiensis*. A checklist of helminths from the badger in North America is included.

There is one comprehensive report, by Erickson (1946), which records six species from the badger, *Taxidea taxus* (Schreber), in Minnesota. Additional scattered records deal with particular species. This prompted us to summarize the literature and study the prevalence of helminths in the badger in north central North Dakota. The parasites recovered are reported herein, together with a checklist (Table 1) of the helminths of *T. taxus* in North America. Presently this host is known to be liable to 17 species.

Materials and Methods

Seventeen badgers from north central North Dakota (Ward, Renville, Mountrail, McLean, and McHenry Counties) were examined for helminths during 1968 and 1969. During the summer of 1970, an additional 44 specimens were examined for cestodes only. The majority of the badgers was shot or trapped by local farmers. Others were either trapped or obtained as fresh road kills by personnel of our laboratory.

The trematodes and cestodes were fixed in A.F.A. or 10% neutral formalin and stained with Ehrlich's hematoxylin or Mayer's acid carmalum. Fast-green in 95% ethyl alcohol was used to counter stain the collar spines of *Euparyphium melis*. Nematodes were fixed in hot 70% ethyl alcohol, cleared in glycerine alcohol, and mounted in glycerine or glycerine jelly.

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Results and Discussion

Nine species of helminths were recovered. These included two trematodes, two cestodes, and five nematodes (Table 2). Of these, *Euparyphium melis*, *Molineus patens*, and *Ascaris columnaris* occur in both North America and Eurasia. The remaining are restricted to the Nearctic region.

Trematoda

Our report of *Alaria* (*Paralaria*) *taxideae* from North Dakota is apparently the third known occurrence of this trematode in the badger. It has previously been reported from the striped skunk, *Mephitis mephitis* (Schreber), in North Dakota by Dyer (1970). It is also known to occur in *M. mephitis*, spotted skunk, *Spilogale putorius* (L.), ermine, *Mustela erminea* Bonaparte, long-tailed weasel, *M. frenata* Lichenstein, and *T. taxus* in Minnesota (Swanson and Erickson, 1946; Erickson, 1946).

Euparyphium melis is common in Eurasian Mustelidae and was first found in North America by Law and Kennedy (1932) in mink, *Mustela vison* Schreber, from Ontario, Canada. It has since been reported from the otter, *Lutra canadensis* (Schreber), and *M. vison* in Michigan and Minnesota (Beaver, 1941), raccoon, *Procyon lotor* (L.), in North Carolina, South Carolina, and Georgia (Harkema and Miller, 1964), and *M. vison* in North Carolina (Miller and Harkema, 1964) and Wisconsin (Dorney and Lauerman, 1969). The occurrence of *E. melis* in North Dakota is a new locality record and constitutes the first report of an echinostome from the badger.

Table 1. Checklist of helminths from the badger in North America.

Helminths	Geographic location and reference
TREMATODES	
Alaria (*Paralaria*) *taxideae* Swanson and Erickson, 1946	Minnesota (Swanson and Erickson, 1946; Erickson, 1946).
Euparyphium melis (Schrank, 1788) Dietz, 1909	North Dakota (present work).
Euparyphium sp.	Minnesota (Erickson, 1946).
CESTODES	
Atriotaenia (*Ershovia*) *procyonis* (Chandler, 1942) Spassky, 1951	North Dakota (present work).
	Wyoming (Keppner, 1969b).
Mesocestoides carnivoricolus Grundmann, 1956	Utah (Grundmann, 1956, 1958).
Monordotaenia taxidiensis (Skinker, 1935) Little, 1967	Colorado (Leiby, 1961).
	Montana (Skinker, 1935).
	North Dakota (Pederson and Leiby, 1969; present work).
	Wisconsin (Rausch, 1947).
	Wyoming (Honess, 1937; Keppner, 1967).
NEMATODES	
Ancylostoma caninum (Ercolani, 1859) Hall, 1913	Arizona (Hannum, 1942).
Ancylostoma taxideae Kalkan and Hansen, 1966	Kansas (Kalkan and Hansen, 1966).
	North Dakota (present work).
Angiocaulus gubernaculatus (Dougherty, 1946) Schultz, 1951	California (Dougherty, 1946).
Ascaris columnaris Leidy, 1856	Colorado (Leiby, 1961).
	Minnesota (Erickson, 1946).
	North Dakota (present work).
Ascaris sp.	Wisconsin (Morgan, 1943).
Filaria martis Gmelin, 1790*	Kansas (Worley, 1961).
	Mexico (Caballero y C., 1948).
Filaria taxideae Keppner, 1969	North Dakota (present work).
	Wyoming (Keppner, 1969a).
Molineus felineus Cameron, 1923	Utah (Grundmann, 1957).
Molineus mustelae Schmidt, 1965	Wyoming (Keppner, 1969b).
Molineus patens (Dujardin, 1845) Petrov, 1928	Minnesota (Erickson, 1946).
	North Dakota (present work).
Monopetalonema? *eremita* Leidy, 1886	Wyoming (Leidy, 1886).
Physaloptera maxillaris Molin, 1860	Minnesota (Erickson, 1946).
	Unknown (Morgan, 1941a).
Physaloptera torquata Leidy, 1886	Arizona (Hannum, 1942).
	California (Morgan, 1942).
	Illinois (Morgan, 1941b, 1942).
	Minnesota (Erickson, 1946).
	Montana (Ehlers, 1931).
	North Dakota (present work).
	Pennsylvania (Leidy, 1886; Walton, 1927; Canavan, 1931).
	Wisconsin (Morgan, 1941b, 1942, 1943).
	Wyoming (Leidy, 1886).
Trichinella spiralis (Owen, 1835) Railliet, 1895	Wyoming or New York (Herman and Goss, 1940).

* Keppner (1969a) states that nematodes reported as *F. martis* by Worley (1961) should be considered conspecific with *F. taxideae* and also questions the identity of *F. martis* of Caballero y C. (1946).

Cestodes

Atriotaenia (*Ershovia*) *procyonis* is a common parasite of raccoons throughout most of southern North America. The first record of its occurrence in the badger is that of Keppner (1969b) from Wyoming. In North Dakota this cestode is frequently found in raccoons (unpublished data) and badgers. This finding establishes a new locality record and is apparently only the second report of *A. procyonis* from the badger.

The distribution of *Monordotaenia taxidiensis*

Table 2. Species of helminths recovered from 17 badgers in north central North Dakota.

Species	Number infected
TREMATODES	
Alaria (Paralaria) taxideae	5
Euparyphium melis	2
CESTODES	
*Atriotaenia (Ershovia) procyonis**	10
*Monordotaenia taxidiensis**	7
NEMATODES	
Ancylostoma taxideae	5
Ascaris columnaris	6
Filaria taxideae	1
Molineus patens	11
Physaloptera torquata	17

* Of the 44 additional badgers examined for cestodes only, six were infected with *A. procyonis* and eight with *M. taxidiensis*.

is limited to North America where it is commonly found in badgers from the northern United States. It has not been recorded from another definitive host.

Nematodes

Five species were recovered. Three of these, *Ascaris columnaris*, *Molineus patens*, and *Physaloptera torquata*, are common in the badger and other Mustelidae from North America. Both *A. columnaris* and *M. patens* have been reported from *M. mephitis* in North Dakota (Dyer, 1970). *P. torquata* represents a new distribution record.

Our finding of *Ancylostoma taxideae* is the only report of this nematode since its original description from a badger in Kansas.

Filaria taxideae was found in the subcutaneous tissue of the thigh of a single badger. The only other mustelid from which this species has been reported is the striped skunk (Keppner, 1969a). Its occurrence in North Dakota constitutes a new distribution record.

Acknowledgments

We wish to express our appreciation to the North Dakota Game and Fish Department for permission to collect badgers, and to Mr. Ernest D. Pederson for technical assistance.

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The Cuticular Ultrastructure of *Paragordius varius* (Leidy, 1851) (Gordioidea: Chordodidae)

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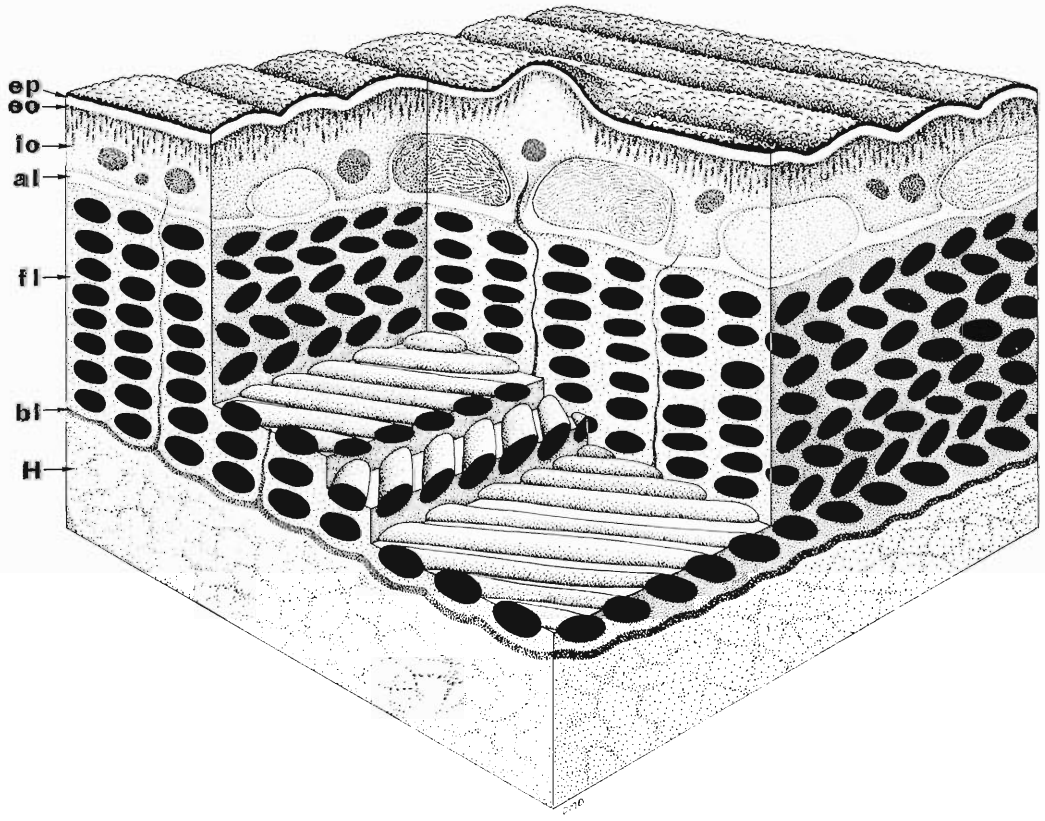
ABSTRACT: The structure of the cuticle of an adult nematomorphan, *Paragordius varius*, has been examined by means of electron microscopy. Present studies reveal several distinct layers and structures previously unresolved within the cuticle. The cuticle apparently possesses morphologically similar structures and layers to those found in various nematode cuticles. The layers (named inwardly) are: ependyma, external cortical, internal cortical, areolar, fibrous, and basal lamella.

The light microscopy of the adult cuticle of *Paragordius varius* was originally done by Montgomery (1903) and was reworked and supplemented by May (1919). May (1919) described the adult cuticle as consisting of an outer homogenous layer with "protoplasmic connections" to the epidermis and with areolae overlying a large fibrous layer. Later, Kirjanova (1959) recognized four layers in the cuticle of all nematomorphans: defense, areolar, fibrous, and pigmental layers. The primary aim of the present study is to reveal the structure of the cuticle of gordioids by means of electron microscopy.

Materials and Methods

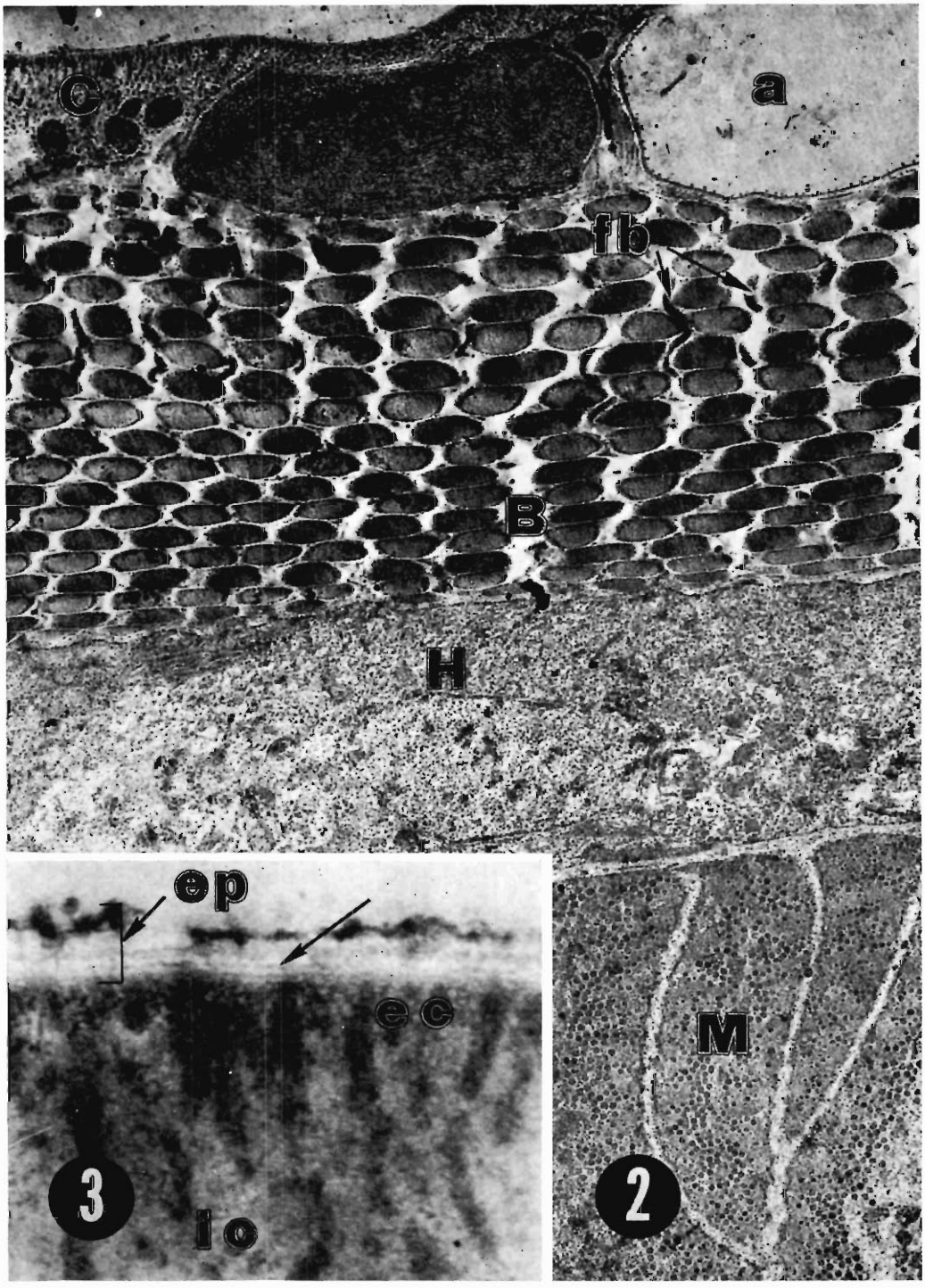
Adult specimens of *Paragordius varius* were collected from the east fork of Clear Creek at



Abbreviations (all figures): a, areole; ab, amembranous body; al, areolar layer; B, basal portion of cuticle; bl, basal lamella, C, cortical portion of cuticle; ec, external cortical layer; ep, ependymal layer; fb, fibrillar bundle; H, hypodermis; ic, internal cortical layer; M, muscle; Ma, macroanal.

Figure 1. A three-dimensional diagram of the adult cuticle of *Paragordius varius*.

the Fallsville Wildlife Area, Highland Co., Ohio. A female specimen was fixed for 12 hr at room temperature in 5% gluteraldehyde-phosphate buffer at pH 7.4, was washed for 1½ hr in phosphate buffer, and while in this wash was cut into pieces. The anterior end, posterior end, and portions of the cuticle were removed and used for the identification of the species. Lengths from the mid-portion of the body were then placed in a phosphate-buffered 1% osmium tetroxide solution at 4 C for 3 hr. These were dehydrated in a graded series of ethanol. Following two changes of propylene oxide (15 min each) the tissue was placed in a graded series of resin concentrations: 3 parts propylene oxide—1 part resin (15 min); 1—1 (1 hr); 1—3 (18 hr); and full strength resin (29 hr). The resin was a modification of Luft's embedding medium (20 ml Epon 812, 20 ml Araldite 502, 60 ml dodecenylsuccinic anhydride, and 2 ml dimethyl phthalate) (Geisy, personal communication). Specimens in the embedding medium were placed in an oven at 75 C for 3 days to effect polymerization of the resin. Sections were cut with glass knives on either a Servall Porter-Blum MT-1 or MT-2 microtome. Several "thick" sections (0.2–0.5 μ were attached to glass slides and stained with hot, aqueous 1% solution of Azure B for preliminary evaluation of the cuticle with a light microscope. Sections were mounted on 200 mesh copper grids coated with 2% parlo-



dion in amyl acetate solution. Sections were counterstained with a saturated solution of uranyl acetate in 50% ethanol (Gibbons and Grimstone, 1960) and in lead citrate prepared according to Venable and Coggeshell (1965) or Reynolds (1963). The cuticle was observed and photographed with an RCA electron microscope Model EMU-3H at both 50 and 100 kilovolts.

Observations

The general arrangement of the cuticle of *Paragordius varius* is seen in Figures 1 and 2. The cuticle can be divided into three main areas: cortical, areolar, and basal. The cortical area (cortex) can then be subdivided into an ependymal, external cortical, and internal cortical layers, while the basal area may be subdivided into a fibrous portion and a basal lamella.

The ependymal layer, outermost layer of the cortex or "garment" of the cuticle, consists of two strata: an outer, irregular osmiophilic stratum and an inner nonstaining stratum. The inner stratum (300–700 A thick) contains moderately dense lamellae (Fig. 3).

Beneath the ependymal layer, the osmiophilic external cortical layer is characterized by its many granular projections into the adjacent internal cortical layer. Many of the cylindrical projections (300–600 A in diameter) apparently end blindly within the internal cortical layer; while others appear to pass through the entire thickness of the internal cortical layer, or into the lamellar covering of the various areolae present in the cuticle.

The lightly granular internal cortical layer makes up the bulk of the cortex. Its internal boundary is well defined (Fig. 1), while the outer border is obscured by the projections of the external cortical layer. At the base of this layer, amembranous osmiophilic bodies are found aligned between and exteriad to the areolae. The amembranous bodies are ovoid to spherical in shape, composed of small granules, and lack an enclosing membrane (Fig. 5).

The areolar layer is bounded externally by the cortex and internally by the basal division of the cuticle. Within this layer three distinct types of areolae have been observed: areolae with large fibrils (Fig. 6), areolae with small fibrils (Fig. 6) and areolae without fibrils in their lumen (Fig. 2). Hollow, branching fibrils 190 A and 115 A in diameter are located within the lumen of the areolae with large fibrils and those with small fibers, respectively (Fig. 6). The apparent suspension of these fibrils within the lumen suggests the presence of an osmiophilic matrix within both bodies. Several pores opening to the exterior were observed on the outer surface of the areolae without fibrils.

All three types of areolae share a similar enclosing lamellar morphology. Typically, there are three osmiophilic lamellae surrounding a body, each of these are in turn separated by a nonstaining lamella (Fig. 6). Often a fourth discontinuous lamella is found on the cortical side of the body. Where the bodies lie close to the surface or receive projections from the external cortical layer, the enclosing lamellae lose their distinct stratification and the osmiophilic material appears to coalesce.

Mesiad to the areolae lies the thickest layer of the cuticle, the fibrous layer. This layer is composed of several strata (16–19 observed in this study), each stratum consisting of a single layer of parallel fibers (Figs. 1, 2). The layers of fibers wind spirally around the body, each layer alternating in one of two directions (May, 1919). The fibers also appear to be suspended in an osmiophobic matrix. Myelin figures (60–130 A thick) are found between the layers of fibers. Individual fibers are composed of a granular substructure arranged in bands (Fig. 4).

Fibrillar bundles and macrocanals have been observed traversing the fibrous and areolar layers of the cuticle. The macrocanals open directly into the internal cortical layer and basally they apparently connect into the hypodermis. These large canal-like structures have always been found to open between two areolae

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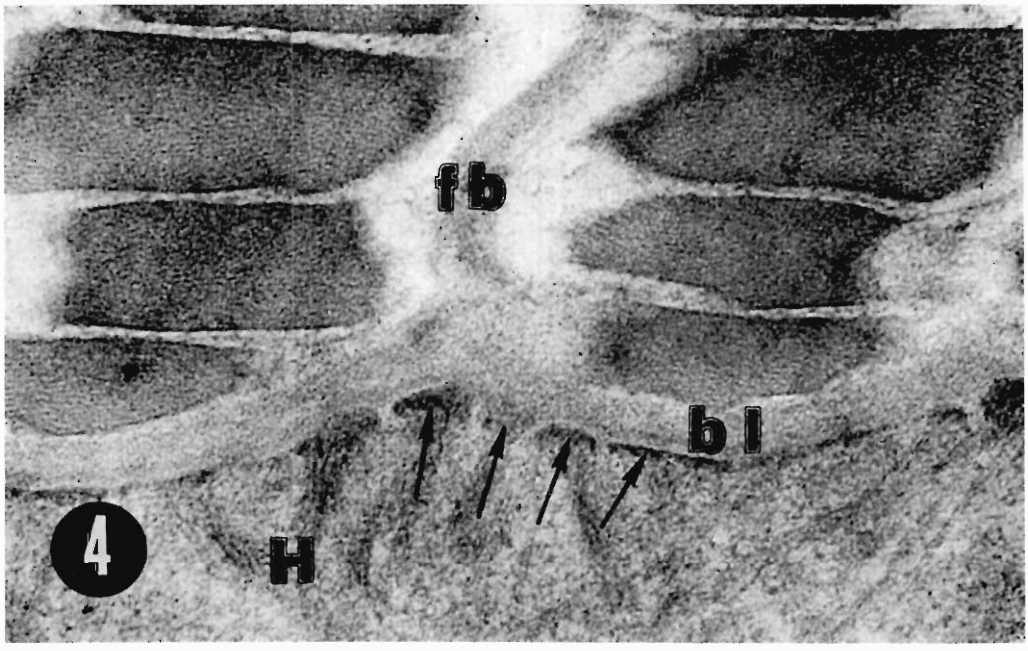
Figure 2. Electron photomicrograph of a cross section through the body wall × 7,300.

Figure 3. Cross section through the cortical layer of the cuticle. The arrow indicates the moderately dense lamellae × 123,750.



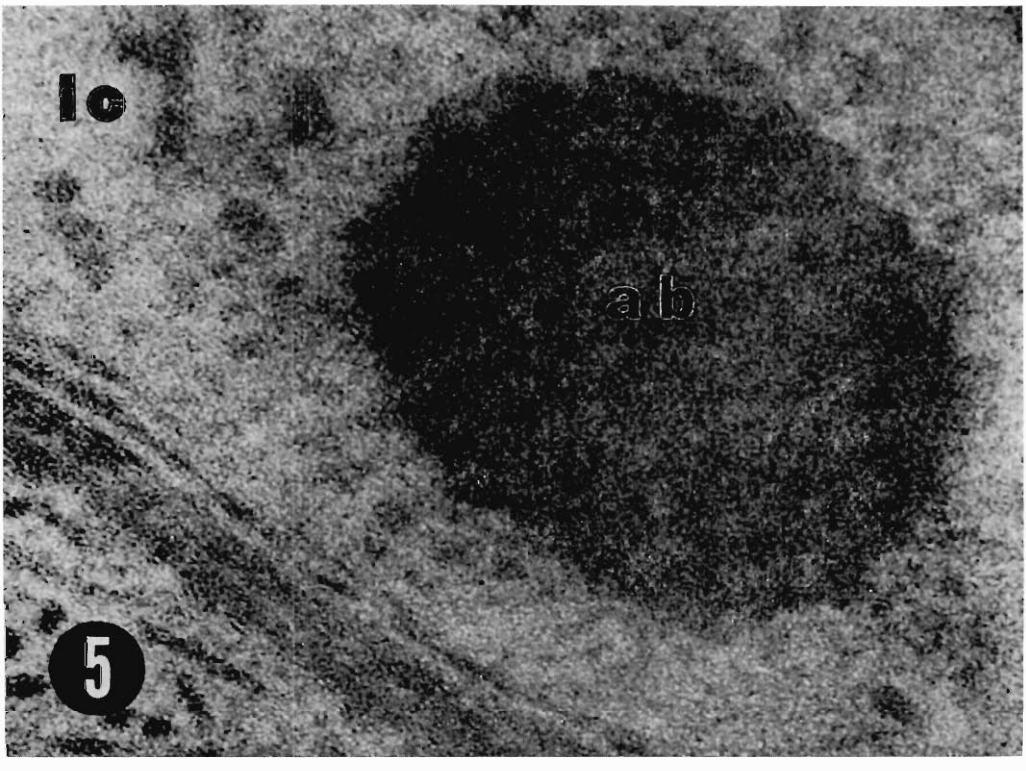


Table 1. A comparison of the suggested divisions for the cuticle of *Paragordius varius* to the cuticle of Nematodes.

May & Montgomery	Kirjanova	Present Study (*P. varius*)	Nematodes (Lee, 1967)
Homogenous layer	Defensive layer	Cortical layer	Cortical layer
		ependymal layer	outer membrane
		external cortical layer	external cortical layer
		internal cortical layer	internal cortical layer
Areolar layer	Areolarnal layer	Areolar layer	Matrix layer
		Basal layer	Basal layer
Fibrous cuticle	Support layer	fibrous layer	fibrous layer
	Pigmental layer	basal lamella	basal lamella

and also are always located beneath a tubercle (Fig. 6). The macrocanals are double-walled and are generally of a larger size (1370–2740 A in diameter) than the fibrillar bundles (830–1900 A in diameter). The fibrillar bundles (Fig. 4) are composed entirely of small fibrils of the same nature as are found in the basal lamella and apparently lack a lumen. Distally, the fibrillar bundles connect into the lower border of the internal cortical layer where they branch to form a fine reticulum of fibrils. Some of the bundles have been observed to connect into the lamellar covering of the areolae. Proximally, the fibrillar bundles apparently arise directly from the basal lamella (Fig. 4).

The basal lamella is composed of fibrils and lies directly over the wavy border of the hypodermis. The upper portions of the hypodermis possesses many hemidesmosomes. Several groups of fibrils, arising from the hemidesmosomes were seen to traverse the hypodermis.

Discussion

Inglis (1964b) states that "nematode cuticle is best considered as a three layered system liable to modification and elaboration around, or in association with a system of punctation canals." Although the cuticle of *P. varius* is distinct from any given nematode, it morphologically fits into the generalized pattern stated above (see Table 1).

The outermost area of gordioid cuticle, called the homogenous layer by Montgomery (1903) and May (1919) and called defensive layer by Kirjanova (1959), has been resolved into three distinct sublayers: ependymal, external cortical, and internal cortical.

Although the outermost layer of nematode cuticle is variously named, the ependymal layer of *P. varius* corresponds best, by virtue of its morphology, to the cuticle-hypodermis membrane of *Nematospiroides dubius*. The external surface of *N. dubius* is limited by a triple-layered membrane, 100 to 135 A thick. Extending from the outer leaflet is a filamentous zone (Bonner, Menefee, and Etges, 1970). The corresponding area in *P. varius* has been named ependyma in order to avoid confusion with the interface between the adult cuticle and the hypodermis.

The cortical sublayers of *P. varius* have a similar osmic staining reaction and positioning to that reported for *Meloidogyne javanica* by Bird and Rogers (1965). The granular material in the lower portion of the "homogenous" cuticle reported by May (1919) and the amembranous osmiophilic bodies observed here are apparently the same. They are of the same shape (spherical) and location (interareolar). No rod-shaped bodies (Montgomery, 1903) were found. These amembranous bodies appear to be composed by small granules and have an osmiophilic staining similar to that of the external cortical layer. In the course of preparing

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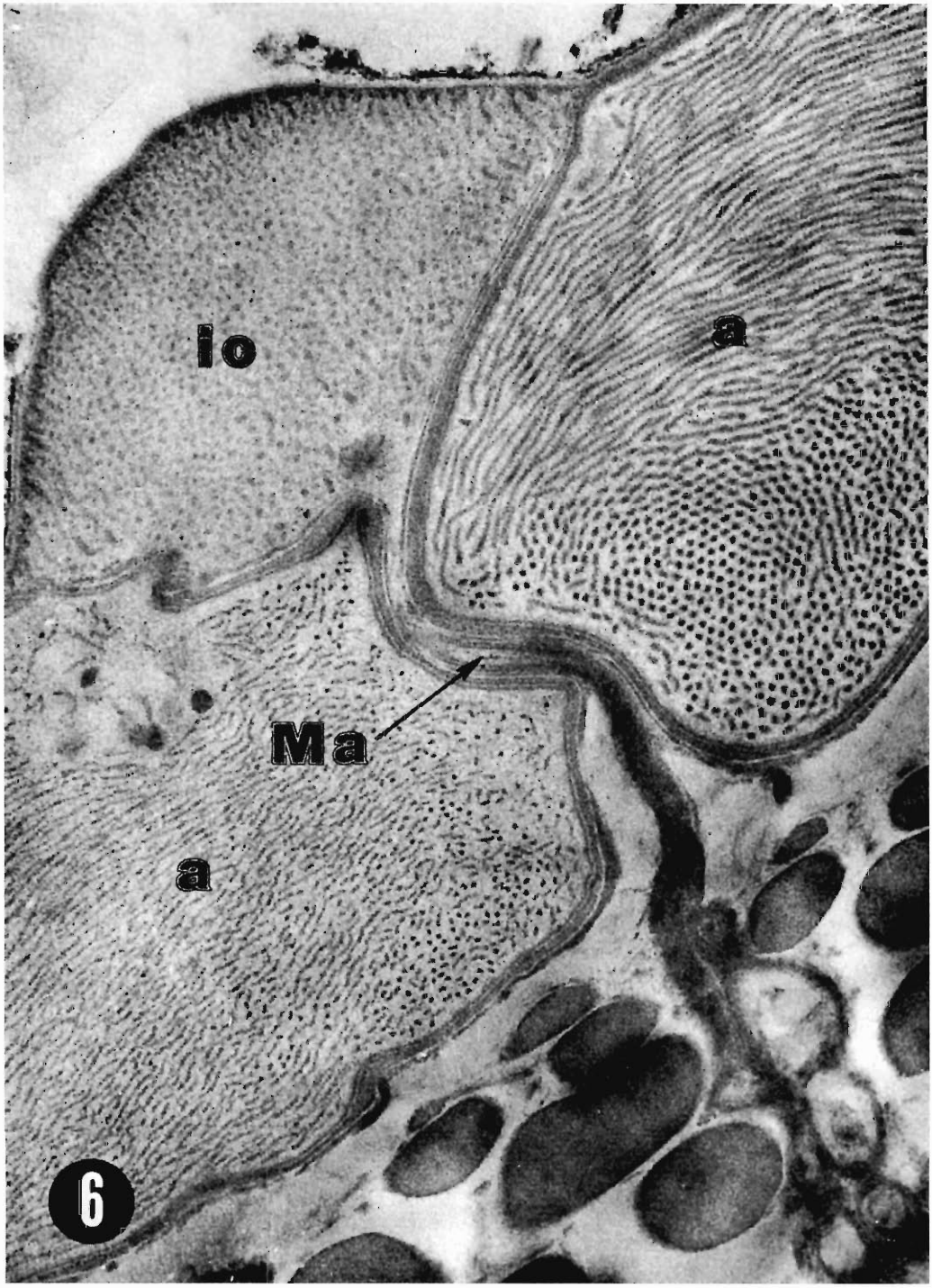
Figure 4. Cross section through the inner portion of the basal area of the cuticle and upper portion of the hypodermis. Arrow indicates hemidesmosomes × 57,750.

Figure 5. Section through the lower portion of the internal cortical layer × 97,500.



slices of cuticle for species identification, it was observed the cuticular pigmentation resides in the upper or cortical area. Since the immature or developing cuticle is white, these bodies may represent the residue of a "tanning" compound secreted into the cuticle.

The areolar layer which is bounded externally by the cortex and internally by the basal layer, corresponds by this position to the matrix layer of nematode cuticle. No structures reported in nematode cuticle apparently correlate directly with the areolae of *P. varius*. Still, the nematodes *Neochromadoria* sp., and *Chromadorella* sp. show cuticular differentiations (lateral bars and hexagonal blocks) arising as modifications of canals (Inglis, 1964b). Three kinds of areolae, rather than the one type previously reported, have been demonstrated. Nonstained cuticle prepared for light microscopy (cleared and mounted in glycerine or Hoyer's media) shows only one type of areole. While preparations stained with osmium, azure B blue or azan's stain reveal both stained and non-stained areolae.

May (1919) cites the presence of "protoplasmic strands" running from the hypodermis to the cuticular surface, usually between the areolae, and cites Vejdovsky's observation of these strands in relation to the areolae of other gordioids. There were several suggestions of fibrillar bundles attaching into the lamellar covering of the areolae. Inglis (1964a) indicates two types of canals or "strands," massive fibrillar elements and punctation canals in the lips of the nematodes *Dujardinascaris* sp., *Porrocaecum* sp., and *Angusticaecum* sp. Two corresponding types of structures have also been demonstrated in this study. The larger macrocanals appear more canal-like, while the fibrillar bundles appear to be entirely composed of fibers. The insertion of the fibrillar bundles into the basal lamella and upper portions of the cuticle suggests a possible anchoring function in the adult.

It has been suggested (Hyman, 1951) that the cuticular ornamentation of gordioids may be sensory in nature. This work does not support this contention. However, a closer examination of the tubercles on the posterior end of males may better serve to clarify the function of these structures.

The basal division of nematode cuticle varies from the complete absence of fibers, reported for the adults of *P. decipiens* by Davey (1965) and *Euchromadora vulgaris* by Watson (1965) to as many as eight layers in some ascarids (Hyman, 1951). In general the larger nematodes possess these layers while the smaller do not (Lee, 1967). In *P. varius*, the number of fibrous layers present exceeds that reported for any nematode. May (1919) has recorded as many as twenty-four present in the cuticle of *P. varius*, while Montgomery (1903) has reported only eleven. This apparent disparity is probably a phenomenon of the area of the body examined. May (1919) reports forty-five layers in the mid-body and thirty in the posterior of *G. robustus*. The corresponding spiral fiber system of nematodes allows the anisometeric stretching (ability to stretch antero-posteriorly but not radially) of the cuticle and is usually considered a refinement of large, highly evolved forms (Inglis, 1964b).

The basal lamella corresponds also to the pigmental layer of Kirjanova (1959) and to the basal lamella of nematode cuticle (Lee, 1967). In the sections observed no pigments or crystals were found. As stated earlier, most of the cuticular pigments appear to reside in the cortical layers.

The hypodermal arrangement of hemidesmosomes and traversing fibrils in *P. varius* corresponds to that reported for *Nippostrongylus brasiliensis* by Lee (1970). It is suggested that this arrangement allows the indirect attachment of the muscles to the cuticle.

Although the cuticle of *P. varius* is remarkably similar to nematode cuticle, other gordioid characters still warrant the present retention of the gordiacea as a group separate from the nematoda.

Acknowledgments

Appreciation to Dr. John L. Crites and Dr. Wayne B. Parrish for their advice and help in the operation of the electron microscope.

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Figure 6. Photomicrograph through tubercle. × 26,000.

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Investigations on the Trematode Fauna of Hyderabad, A.P. Part II. Parasites of Birds—(C). *Psilochasmus singhi* sp. n. from a Common Whistling Teal, *Dendrocygna javanica*

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ABSTRACT: *Psilochasmus singhi* sp. n., is described from the Whistling Teal, *Dendrocygna javanica* from Hyderabad, A.P., India and compared with the other three previously described Indian forms and also with a Russian and a W. German species. It differs from all other species of the genus in the position of its genital pore, disposition of the ovary and vitellaria, structure of the esophagus, and the principal measurements of the body.

The genus *Psilochasmus* was established by Lühe in 1909 with (1). *P. oxyuris* (Creplin, 1825) as its type species. In addition to it the following 9 forms have been described so far from different parts of the world:—

(2). *P. longicirratus* Skrjabin, 1913 from the intestine of the white-eyed Pochard, *Fuligula nyroka* in Russian Turkestan.

(3). *P. agilis* Travassos, 1921 from *Poecilonetta bahamensis* in Brazil.

(4). *P. lecithosus* Otte, 1926 from the intestine of domestic duck, in Latvia.

(5). *P. japonicus* Ishii, 1935 from the intestine of wild domestic duck, *Fuligula nyroca* from Japan.
(6). *P. skrjabini* Gnedina, 1946 from *Nyroca rufa* from Azerbaidzhan SSR.
(7). *P. alii* Jaiswal, 1957 from the intestine of a Comb-Duck, *Sarkidiornis melonotus* from Hyderabad.
(8). *P. megacetabulus* Jaiswal, 1957 from the intestine of *Ardeola grayi* from Hyderabad.
(9). *P. indicus* Gupta, 1957 from Brahmi duck, *Casarca rutila* from Allahabad.
(10). *P. aglyptorchis* Loos-Frank, 1968 from the intestine of an experimental Herring Gull, *Larus argentatus* in W. Germany.

P. oxyuris (Creplin, 1825) Lühe, 1909 was redescribed by Odhner (1913) and was recorded on several occasions by Baugh (1949), Singh (1954), and others. *P. agilis* Travassos, 1921 was regarded a synonym of *P. oxyuris* by Gupta (1957), due to their close resemblance in the shape of the body and general topography of the organs, lobed structure of the testes, extent of cirrus sac, position of the genital pore, and distribution of vitellaria. Inamdar and Bhalerao (1944) had also expressed doubt about the validity of *P. agilis*.

P. longicirratus Skrjabin, 1913 was recorded by Tubangui (1932), Hsü and Chow (1938), Yamaguti (1939), and Inamdar and Bhalerao (1944). In 1939, Yamaguti regarded *P. japonicus* Ishii, 1935 as synonymous with *P. longicirratus*. Stunkard and Dunihue (1931) pointed out the synonymy of *P. longicirratus* and *P. oxyuris* on the basis of the varying length of the cirrus sac and their conclusion in suppressing *P. longicirratus* was also supported by Singh (1954).

P. lecithosus Otte, 1926 has been regarded by Baylis (1932) as identical to an echinostome species of the genus *Hypoderaeum* Dietz, 1909.

Psilochasmus singhi sp. n.

In November, 1961 two specimens of this fluke were obtained from the intestine of the Common Whistling Teal, *Dendrocygna java-*

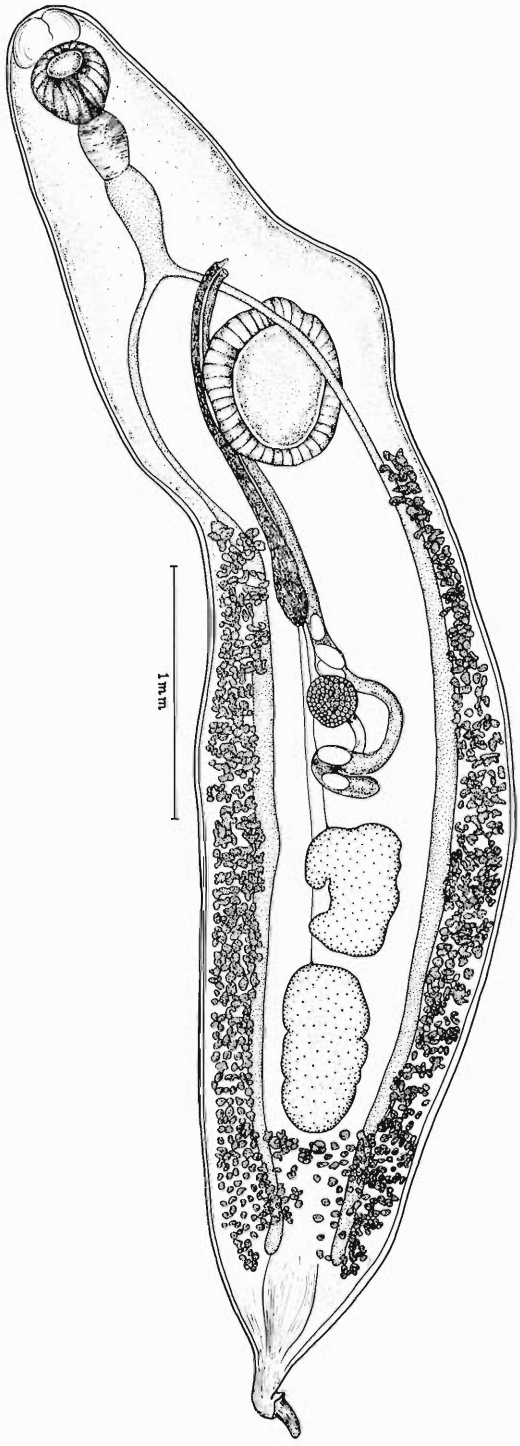

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Figure 1. ***Psilochasmus singhi*** **sp. n. Dorsal view.**

nica. A detailed examination of the parasite and the study of the relevant literature revealed that it constitutes a new species of the genus *Psilochasmus*.

The body of the fluke is elongated and spindle-shaped with a bluntly rounded anterior end and a sharply marked off retractile tail bearing a terminal spine. The body is uniformly elongated without any marked difference between the pre-acetabular and the post-acetabular regions of the body, as is commonly found in other species of this genus. At the hind-most region of the body a bundle of muscles converge posteriorly to retract the pointed tail partially into a sac-like sheath. The flukes measure 4.806–6.083 mm in length and 0.839–1.258 mm in greatest width. The oral sucker is sub-terminal and spherical in outline, measuring 0.303–0.322 by 0.296–0.303 mm. The acetabulum is strongly muscular and rounded in shape. It measures 0.477–0.658 by 0.465–0.580 mm and is nearly double the size of the oral sucker, being separated from it by a distance of 0.968–1.079 mm. The body is unarmed and the cuticular covering is smooth. The oral sucker surrounds the mouth which communicates with the pharynx by means of a very short pre-pharynx 0.194–0.232 mm in length. The muscular pharynx is fairly large and elongate measuring 0.245–0.277 by 0.168–0.187 mm, it is followed by a long and stout esophagus which is 0.439–0.568 by 0.110–0.174 mm. It bifurcates into two intestinal crura which pass along the sides of the body touching the inner margins of the vitelline follicles, terminating posteriorly somewhat above the level of the vitelline glands at about 0.503–0.774 mm from the tip of the tail.

The excretory bladder is Y-shaped and opens to the exterior at the excretory pore placed at the base of the caudal spine.

The testes are approximated and placed one behind the other in the post-equatorial region of the body, they are slightly notched, measuring 0.458–0.529 by 0.258–0.374 mm and 0.490–0.664 by 0.264–0.348 mm, respectively. The cirrus sac is very elongate and stretches from slightly above the level of the ovary to much above the level of the acetabulum, so as to open at the genital pore which is situated slightly behind the level of the intestinal bifurcation. The cirrus sac encloses a seminal vesicle, pars prostatica, and a well developed cirrus which is found protruding from the genital aperture.

The ovary is equatorial in position and is placed much above the anterior testis. It is somewhat rounded in outline measuring 0.174–0.213 by 0.168–0.206 mm. The shell gland is present and Laurer's canal is discernible whilst the receptaculum seminis is lacking. The oviduct originates from the posterior border of the ovary and continues into the uterus which forms a few loops in the space between the ovary and the anterior testis. The metraterm is strongly muscular and courses parallel to the cirrus sac so as to open immediately posterior to the male genital pore. The vitellaria are follicular and extra-cecal in position and are spread over in the lateral zones of the body. They originate somewhat behind the level of the acetabulum and extend posteriorly slightly beyond the tips of the ceca. Posteriorly the follicles of both sides merge in the middle immediately behind the hind testis but in the anterior region of the body near the acetabulum the follicles of the right and left sides are quite distinctly separated from one another. The eggs are thick-shelled measuring 90–142 × 70–72 μ and are very few in number.

DISCUSSION: The form described herein differs from all the known species in the position of its genital pore, disposition of the ovary and vitellaria, structure of the esophagus, and the principal measurements of the body. It differs from *P. skrjabini* Gnedina, 1946 and *P. aglyptorchis* Loos-Frank, 1968 in the presence of a very prominent esophagus which is completely absent in the former and in possessing a very distinct characteristic horny posterior process which is lacking in the latter.

The form under study also differs from both the species described by the senior author, namely *P. alii* and *P. megacetabulus* Jaiswal, 1957 not only in the position of its genital pore and location of the ovary but also in the length and structure of the esophagus and the pharynx. It also differs from the above forms in the principal measurements of the body.

The form described herein resembles to some extent *P. indicus* Gupta, 1957 but however, differs markedly from it in the disposition, structure, and extent of its vitellaria, the far forward position of the ovary, the location of the genital pore, and also in the length and very stout structure of its esophagus. The vitel-

laria in the form under study extend anteriorly almost up to the level of the acetabulum and the follicles of both the sides are distinctly separated from one another in front of the ovary or behind the ventral sucker. The vitelline follicles in *P. indicus* Gupta, 1957 are restricted anteriorly far behind the ventral sucker and the follicles of the two sides also meet each other in front of the ovary or behind the ventral sucker. Moreover, the ovary in the specimen described herein is equatorial in position whereas it is definitely post-equatorial in *P. indicus*. The genital pore in the species from Hyderabad is placed at about the level of the intestinal bifurcation whilst it is much above that level in *P. indicus*. The pharynx is elongate and the esophagus is comparatively short but very stout in the specimen described herein, while the pharynx is rounded in *P. indicus*. Moreover, the esophagus in Gupta's form is very long and much narrower. Both the forms also differ in the principal body measurements.

Hence in view of the facts mentioned above, it has been found necessary to establish a new species for this form. It is proposed to be named as *Psilochasmus singhi*, in honor of Dr. S. N. Singh.

SPECIFIC DIAGNOSIS: Body elongate and spindle-shaped 4.806–6.083 by 0.839–1.258 mm, anterior end rounded and a sharply marked off retractile tail with a terminal spine; oral sucker 0.303–0.322 by 0.296–0.303 mm; acetabulum 0.477–0.658 by 0.465–0.580 mm, prepharynx very short, pharynx 0.245–0.277 by 0.168–0.187 mm, esophagus 0.439–0.568 by 0.110–0.174 mm; intestinal ceca along the sides of the body; the vitellaria extend anteriorly almost up to the level of acetabulum and the follicles of both the sides are distinctly separated from one another; testes one behind the other 0.458–0.529 by 0.258–0.374 mm and 0.490–0.664 by 0.264–0.348 mm, respectively; ovary equatorial in position, rounded in outline 0.174–0.213 by 0.168–0.206 mm; genital pore slightly behind the level of the intestinal bifurcation; eggs 90–142 by 70–72 μ.

HOST: *Dendrocygna javanica*.

HABITAT: Intestine.

LOCALITY: Hyderabad, A.P.

The type specimen has been deposited in the Helminthological Museum of the Department of Zoology, Osmania University.

Acknowledgment

The authors are deeply indebted to Dr. S. N. Singh, D.Sc. (London), F. N. I., Prof. and Head, Department of Zoology, Osmania University, for providing all the facilities in completing this work.

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The Micro-ecology of Three Species of Monogenetic Trematodes of Fishes from the Beaufort–Cape Hatteras Area[1]

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ABSTRACT: Three species of fishes, *Urophycis regius*, *Stenotomus chrysops*, and *Orthopristis chrysopterus* were found to be parasitized by *Diclidophora maccallumi*, *Microcotyle stenotomi* and *Pseudotagia cupida*, respectively. A fourth species of fish, *Peprilus triacanthus*, was not found to be parasitized. The branchial baskets of each species of fish were divided into arbitrary regions and the number of parasites in each region was determined. Site specificity was determined by application of Chi-square tests to the data. *Diclidophora maccallumi*, the only parasite to occur in sufficient number to be tested, showed site specificity. The specific sites of attachments were correlated with the mechanisms of branchial irrigation, and it was suggested that indicated site specificity may be the result of the force and direction of the gill ventilating current.

Early workers in the field of parasitology noticed that some parasites have a higher affinity, or a specificity, for certain parts or regions of the body than others. Workers such as Cerfontaine (1896, 1898) and Gröben (1940), studying the monogenean genera *Diclidophora* and *Dactylogyrus* respectively, found that members of these genera were consistently found on certain areas of the gills. Frankland (1955) studied *Dactylocotyle denticulata* (Olsson, 1876) Yamaguti, 1963 and confirmed Cerfontaine's findings. She further suggested that young specimens of *D. denticulata* are capable of limited movement on the gills, but that this ability decreases with the age of the parasite. Llewellyn (1956) found that the parasites of seven of eleven species of fishes exhibited a site specificity for particular gill arches. He suggested that the upstream position of the diclidophorid posthaptor and the asymmetry of the posthaptor of the diclidophorid *Anthocotyle merlucci* van Beneden et Hesse, 1863, were adaptations to reduce the resistance of these parasites to the gill ventilating currents. Later works by Llewellyn and Owen (1960) on *Discocotyle sagittata*, Owen (1963) on *Diplozoon paradoxum* and Slinn (1963) on *D. sagittata* supported Llewellyn's 1956 findings. Akazaki (1965) working on *Heteraxine heterocerca*, Wiles (1968) working on *D. paradoxum* and Ktari (1969) working with *Microcotyle salpae* further defined these specific areas of attachment by dividing each gill arch into several arbitrary regions. The parasite's position was then indicated with respect to the assigned regions.

The purpose of this study was to further investigate the distribution of monogenean parasites on the gills of their hosts.

Methods and Materials

Host specimens *Urophycis regius* (Walbaum) (Gadidae), *Stenotomus chrysops* (Linnaeus) (Sparidae), *Orthopristis chrysopterus* (Linnaeus) (Pomadasyidae) and *Peprilus triacanthus* (Peck) (Stomateidae) were collected on the continental shelf between Cape Hatteras and Beaufort, North Carolina from November 10–13, 1969 aboard the R/V EASTWARD (Duke University, N. C.). Ten 30-min otter tows were made with a 16-foot try-net (Table 1). Host specimens were identified on board by Dr. J. A. Musick of the Ichthyology Department of the Virginia Institute of Marine Science (VIMS). After fork length measurements of each fish were taken the gills were removed, wrapped in individual gauze packages, and preserved in a solution of 70% ethanol plus 5% glycerol.

In the laboratory the gills were examined for monogeneids with a stereo-microscope and the exact location of the trematodes recorded before removal for identification. A few selected parasites were photographed *in situ*. To indi-

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Table 1. Stations of the R/V EASTWARD at which fishes were taken.

Station No. DUML	Trawl station location Long. (N)	Lat. (W)	Depth in meters	Specimens taken	No.
13245	34°30′	76°44′	21	*P. triacanthus*	10
13276	35°23′	74°55′	100	*U. regius*	13
13279	35°26′	75°03′	30	*U. regius*	3
13282	35°26′	75°20′	18	*U. regius*	8
13285	35°06′	75°17′	53	*U. regius*	1
13290	34°57′	75°19′	182	*U. regius*	10
13293	35°03′	75°23′	51	*U. regius*	1
13300	34°51′	75°49′	30	*S. chrysops*	14
13307	34°26′	75°30′	30	*O. chrysopterus*	10
				U. regius	3
13309	34°27′	76°21′	26	*U. regius*	3

cate the positions of the parasites it was decided to use arbitrary divisions of the gill arches adopted by Wiles 1968 (Fig. 1). Gill arches were numbered from 1–4 anteroposteriorly. Each arch was divided into three equal sections, dorsal, middle and ventral, whereas each holobranch was subdivided into medial and lateral hemibranchs. The surfaces of the hemibranchs were next designated a) inner, that surface lying between two hemibranchs of the same holobranch; and b) outer, that surface lying between two separate holobranchs. The gill filaments were also equally divided into proximal, middle and distal portions.

For identification the monogenetic trematodes were stained in either Reynolds' Double Stain or Harris' Haematoxylin and mounted in Euperal (Turtox). Original descriptions were used for identifications and the current taxonomic status for each species is in accordance with Yamaguti, 1963.

The Chi-square test was applied to the data to determine if the parasites occurred on one region of the gill more than another. A Chi-square test was made between all arch subdivisions, regions of the arches, surfaces of the hemibranchs and divisions of the filaments unless specificity was obvious or small sample size made it impossible to test.

Results and Discussion

Seventy-six specimens of fishes were collected representing four species. Three of the four species, *Urophycis regius*, *Stenotomus chrysops* and *Orthopristis chrysopterus*, were parasitized by *Diclidophora maccallumi* (Price, 1943) Sproston, 1946, *Microcotyle stenotomi* Goto, 1900, and *Pseudotagia cupida* (Hargis, 1956) Yamaguti, 1963, respectively. *Peprilus triacanthus* was not infested. In Table 2 the total number of individuals, the number of infested individuals, and the infestation rates are given for each species of fish. *U. regius* was most heavily parasitized.

Table 3 lists the morphological regions of the gills indicated in Figure 1, and gives the number of parasites of each species that was recovered from each region. Sample sizes for *M. stenotomi* and *P. cupida* were too small to apply a Chi-square test. *D. maccallumi* occurred in large enough numbers so that tests could be applied.

Chi-square tests indicated that site specificity existed at the 95% level of confidence at some of the regions (Table 4). The numbers of *D. maccallumi* occurring on arches I, II, and III are significantly higher than on arch IV. Since the difference in the numbers of parasites between gill arches I and III was large (Table 3), and the sample size was small, they were tested at the 90% level of confidence. The specificity at this level was narrowed to gill arches II and III. It is possible, that with a larger sample, a

Table 2. The percent of hosts infested and the mean number and range of parasites per host.

Host	Total No. of hosts	No. hosts infested	Infection rate % inf./tot.	Parasite	Total No. parasites	Mean No. para./host	Range of para./host
Urophycis regius (spotted hake)	42	20	47.6	*D. maccallumi*	166	8.3	1–14
Stenotomus chrysops (scup)	14	5	35.0	*M. stenotomi*	7	1.4	1–3
Orthopristis chrysopterus (pigfish)	10	1	10.0	*P. cupida*	1	1	—
Peprilus triacanthus (butterfish)	10	—	—	—	—	—	—

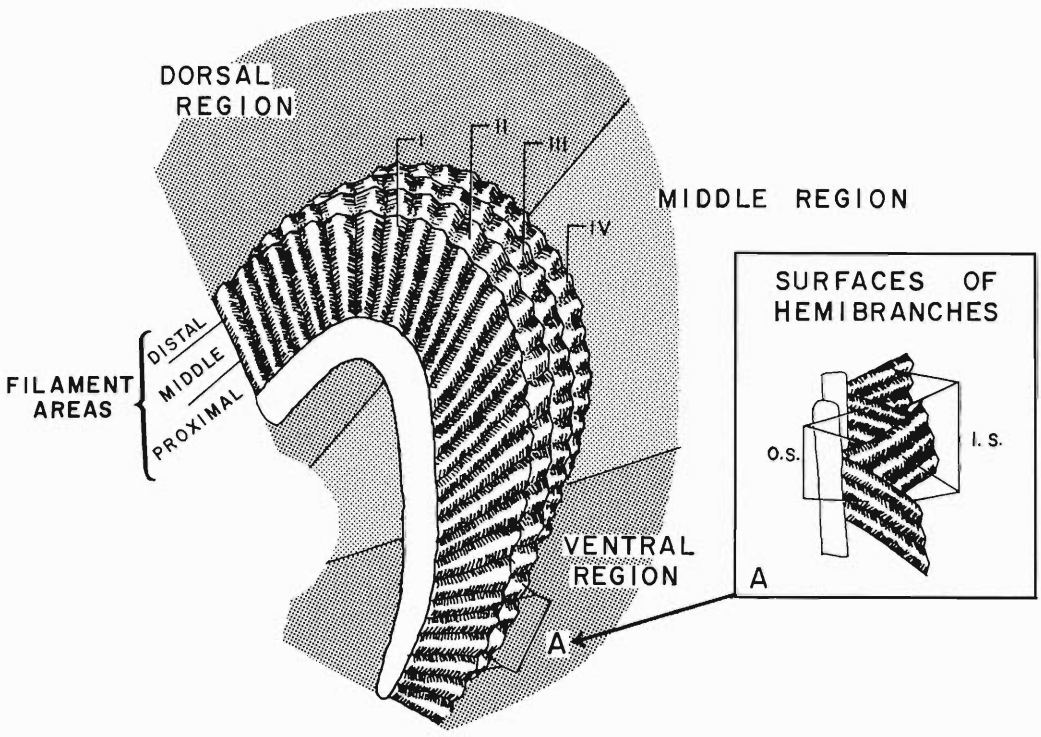

Figure 1. Illustration of the left side of the branchial basket showing the arbitrary divisions. O.S.—outer surfaces; I.S.—inner surfaces.

specificity for arches II and III would be indicated at the 95% level of confidence.

Site specificity is indicated for the middle and lower regions of the gill arches (Table 4). Since 80% of the attached specimens occurred on the inner surfaces of the hemibranchs specificity was obvious and no Chi-square tests were applied. Tests were applied to the inner surfaces of the lateral and medial hemibranchs but no significant differences were indicated (Table 4). Therefore, in all cases, the inner surfaces were "preferred" irrespective of the hemibranch.

Three times as many flukes were found on the middle region of the gill filament as on either the proximal or the distal regions. *D. maccallumi* was generally oriented with its posthaptor located proximally on the filament, and its haptoral clamps attached to lamellae on opposite sides of the same filament, apparently lying parallel to the filament in life (Figs. 2 & 3).

Llewellyn (1956) indicated that *Diclidophora merlangi*, from *Gadus merlangus*, occurred most often on gill arch I, and that *D. luscae*, from *G. luscae*, was more prevalent on gill arches II and III. These parasites were found with their posthaptors upstream to the ventilating current. Frankland (1955) indicated that *Dactylocotyle denticulata*, from *G. virens*, was more prevalent on the inner surfaces of the hemibranchs of gill arch I. Wiles (1968) found that *Diplozoon paradoxum* occurred most often on gill arches I and II in the bream (*Abramis brama* L.), on the inner hemibranch in the bream and minnow (*Phoxinus phoxinus* L.), and on the middle region of the gill arch in the minnow and roach (*Rutilus rutilus* L.). The adhesive attitude and site specificity of *Diclidophora maccallumi* are

Table 3. The distribution of parasites on the gills. Columns headed by a question mark (?) indicate numbers of parasites from undetermined locations.

Parasite	Total	No. of parasites on gill arches					No. of parasites on region of gill arch				Surface of hemibranchs Median		Surface of hemibranchs Lateral			Region of gill filament			
		I	II	III	IV	?	Dorsal	Middle	Ventral	?	In	Out	In	Out	?	Proximal	Middle	Distal	?
D. maccallumi	166	33	55	48	18	12	24	67	50	25	64	11	45	16	30	27	94	20	25
M. stenotomi	7	2	0	4	0	1	1	3	2	1	5	1	0	0	1	4	2	0	1
P. cupida	1	0	1	0	0	0	0	1	0	0	—	—	side		—	0	1	0	0

Table 4. The areas tested for *D. maccallumi* and the Chi-square values. Tests were run using the degree of freedom indicated in the parentheses at the 95% level of confidence.

Areas tested	Chi-square	Calculated Values
Gill arches I, II, III, IV	(3-df)	20.22*
Gill arches I and II	(1-df)	5.50*
Gill arches I and III	(1-df)	2.78
Gill arches I and IV	(1-df)	13.89*
Gill arches II and III	(1-df)	0.48
Gill arches II and IV	(1-df)	17.91*
Gill arches III and IV	(1-df)	13.66*
Regions of gill arch Dorsal, middle, and ventral	(2-df)	24.79*
Regions of gill arch Dorsal and middle	(1-df)	20.32*
Regions of gill arch Dorsal and lower	(1-df)	9.14*
Regions of gill arch Middle and lower	(1-df)	2.47
Hemibranchs Lateral and medial	(1-df)	3.82
Hemibranchs Inner lateral and inner medial	(1-df)	3.30

* Indicates significant difference.

similar to those described by Frankland (1955) for *Dactylocotyle denticulata*, Llewellyn (1956) for *Diclidophora merlangi*, and by Wiles (1968) for *Diplozoon paradoxum*, and may be influenced by the gill ventilating current as Llewellyn (1956) suggested.

An examination of the environmental factors influencing the microhabitat of *Diclidophora maccallumi* may yield a better understanding and possible explanation for the apparent site specificity. Hughes and Shelton (1957, 1958) working with *Salmo trutta* L., *Leuciscus rutilus* L., *Tinca tinca* L., and Saunders (1961) working with *Catostomus commersoni* (Lacépède), *Ictalurus nebulosus* (LeSueur), and *Cyprinus carpio* L. measured the hydrostatic pressure changes of the branchial pump during the respiratory cycle of these fishes. They found that during each cycle the flow of water from the buccal cavity to the opercular cavity was almost continuous and that for only a brief period during each cycle a back-pressure developed, reversing the direction of flow. Bijtel (1949), working with 12 species representing eight families of fishes, indicated that hemibranchs were spread during the respiratory cycle and the tips of hemibranchs on adjacent gill arches touched. He also described a coughing action which occurred periodically during the cycle. During this action muscles in the filament contracted pulling the hemibranchs together, the operculum was closed rapidly, and water was flushed backwards through the

Figure 2. A dorsal view of *D. maccallumi* attached to the inner surface of a hemibranch of *U. regius*. The posthaptor is attached proximally and the body lies parallel to the filament (preserved specimen).

Figure 3. A view of the haptoral clamps of *D. maccallumi* as they appear from the outer surface of the hemibranch of *U. regius* (preserved specimen).

gills. This action apparently served a cleaning function.

The direction of the ventilating current and the position of the hemibranchs during respiration may influence the position of *D. maccallumi* on the gills. The number of animals attached to the outer surface of the hemibranchs was small. This was possibly the result of the coughing action. This coughing action would tend to remove both young attaching forms and adults. If the invasion route of the parasite were passive, through the mouth, the brief backwash period in each respiratory cycle would offer an opportunity for new forms to attach to the exposed inner surface on the hemibranch. If the invasion route were active, through the operculum against the ventilating current, the spread inner surfaces of the gills would be the first surfaces encountered. In either case, the filaments of the hemibranchs appear to be capable of providing some protection from the almost continuous force of the ventilating current. The adduction of the hemibranchs during the coughing action might offer additional protection to those animals attached to the inner surfaces of the hemibranchs. Therefore, the inner surfaces of the hemibranchs would appear to be the more favorable site of attachment.

The fact that *D. maccallumi* occurs on the outer surfaces of the hemibranchs at all seems unusual. Llewellyn and Tully (1969) indicated that *D. macruri* is the only other *Diclidophora* studied that occurs on these outer surfaces. The unusual ability of *D. maccallumi* and *D. macruri* to attach to these surfaces may be accounted for by the structure of their posthaptoral clamps. A detailed study of these clamps and their method of attachment is necessary to gain a greater understanding of this phenomenon.

Diclidophora maccallumi occurred most often on gill arches I, II, and III. Paling (1968) working with *Salmo trutta* L., using glochidia of *Anodont acygenea* as indicators, determined

that the greatest volume of water in the gill ventilating current passed over the second and third gill arches. The first gill arch received the next greatest volume and the fourth the least. The distribution of *D. maccallumi* on the gill arches appears to vary directly with the distribution of the volume of the gill ventilating current. Apparently the greater volume of water flowing over the first three gill arches gives more parasites the opportunity to attach to these gill arches.

The indicated specificity for the middle and lower regions of gill arches could be attributed to the morphology of the branchial basket and the ventral position of the opercular opening. Larvae could come in contact with the lower region first during the brief backwash period and, therefore, would occur in high numbers in the middle and lower regions.

A higher number of *D. maccallumi* occurred on the middle region of the filament. This could have resulted from a need to maintain the mouth in a feeding position at the distal end of the filament as Frankland (1955) suggested for *Dactylocotyle denticulata*. The upstream position of the haptor and the resulting body position, seemingly parallel to the gill filament in life, may be, as Llewellyn (1956) suggested, an obvious adaptation that reduces resistance to the gill ventilating current.

Limited mobility of *Diclidophora* larvae (Frankland, 1955) and the knowledge resulting from this study would seem to indicate that any gill site specificity of *Diclidophora maccallumi* is primarily the result of the force and direction of the ventilating current and not selection on the part of the parasite. This is in accord with a similar suggestion made by Llewellyn (1956).

It must be remembered that only some of the physical factors which seem to influence site specificity have been discussed here. Physiological, behavioral, and further ecological studies are needed to provide a more complete picture of this type of host–parasite relationship.

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Comparative Development of *Ascaris suum* in Rabbits, Guinea Pigs, Mice, and Swine in 11 Days

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ABSTRACT: A comparative study was made of the development and migratory patterns of *Ascaris suum* in mice, guinea pigs, rabbits, and swine. Host animals were each given a single dose of 1,300 infective eggs and then killed 1, 2, 3, 4, 7, 9, or 11 days after infection (DAI). In mice, the infection essentially terminates 4 DAI with the attainment of middle third-stage in the liver, although few larvae migrate to the lungs where a few advance to late third stage. In guinea pigs, significant numbers develop to late third-stage but no farther in the lungs 7 DAI and very few migrate to the intestine. In rabbits, development was practically identical to that in swine in that early fourth-stage appeared in the intestine 11 DAI.

In previous papers on morphogenesis of *Ascaris suum* to the fourth stage in swine (Douvres, Tromba, and Malakatis, 1969) and in vitro (Douvres and Tromba, 1970) we discussed the comparative development of *A. suum* in normal versus abnormal situations. These studies and some observations on the development of *A. suum* in rabbits (Douvres and Tromba, 1966; Douvres et al., 1969) mice, and guinea pigs, led us to conclude that stage identification based on size, location in the host, or number of days of development was unreliable.

Our survey of some recent papers illustrates the confusion arising when the above criteria have been variously interpreted by different investigators. That is, larvae recovered from the lungs of guinea pigs were identified as follows: By depending on body lengths of less or more than 500 μ, larvae were, respectively, second and third stages, 4 or 5 days after infection (DAI) (Soulsby, 1961). By depending on location, larvae were third stage 8 DAI (Saz et al., 1968), and third and fourth stages 7 and 8 DAI (Matov and Terzijski, 1968). In mice, Sinha (1967) characterized as second stage all larvae measuring less than 305 μ, and as third stage, those measuring 315 to 1,960 μ. He found that larvae remained in second stage in the liver up to 3 DAI and were in second and third stages in both the liver and lungs from 4 to 12 DAI. Bindseil (1970) identified all larvae recovered from the lungs of mice up to 4 DAI as second stage; and those recovered from the

same location from 5 to 9 DAI which measured over 500 μ, as "third to fourth stage." Guerrero and Silverman (1969), classified larvae recovered from the lungs of mice 7 DAI as "late third and early fourth stages" depending on location. Williams and Soulsby (1970), again depending on location, identified all larvae recovered from the lungs of rabbits 7 DAI as third stage.

This confusion in identification of *A. suum* larval stages that develop in abnormal hosts was the primary reason for undertaking the present study. Accordingly, we report herein a study comparing the development of *A. suum* in mice, guinea pigs, and rabbits, up to 11 days. The larval stages recovered from these hosts were identified by using the features previously described by us (Douvres et al., 1969) for *A. suum* larvae that developed to fourth stage in the normal host. We originally intended that our previous work with the normal host would serve as the standard for comparison. However, since the infecting dose and the source of eggs in the present study differed from those of the previous one, we included a series of swine as a control.

Materials and Methods

I. Experimental animals

Seven 4- to 6-wk.-old helminth-free Hampshire pigs, 35 male New Zealand white rabbits (1,300–1,500 g), 35 male guinea pigs (300–500 g), and 35 male General Purpose Swiss mice (25–30 g) were used. Pigs, rabbits, and guinea pigs were born and raised at this Laboratory; but mice were obtained from the Rodent and Rabbit Production Section, Laboratory Aids Branch, Division of Research Services, National Institutes of Health, Bethesda, Maryland. The pigs were housed in concrete-floored pens to preclude extraneous helminth infections. The rabbits were housed individually, and the mice and guinea pigs were housed in groups of 5 in wire cages. All animals were fed a balanced ration ad lib.

II. Animal infections

A. suum eggs were collected from the uteri of adult worms (Costello, 1961) then decoated, embryonated (Costello et al., 1963), and stored in 2% formalin in tap water for 1 week to 6 months at 5 C before use. This stock of eggs was used to inoculate all animals. Artificially hatched eggs from this stock released motile larvae that were either enveloped in the sheath of the first molt or had completed the first ecdysis at hatching. Such larvae resembled those in the infective egg described by Alicata (1936) and Douvres et al. (1969).

After eggs were washed 8 times in tap water, the number that contained motile larvae was estimated by counting an aliquot. The volume was adjusted to contain 1,300 eggs/ml, the single dose given to each animal. The eggs were administered orally to pigs, rabbits, and guinea pigs, through a cannula attached to a syringe, and to anesthetized mice through a stomach tube.

The dose of infective eggs, used herein, was determined after a suggestion by Dr. Vassilios Theodorides of Smith, Kline, and French Laboratories, Philadelphia, Pennsylvania. He found that a single dose of 1,000 to 2,000 eggs was optimal for obtaining patent infections of *A. suum* in rabbits. Since his low-dose was to be used for our rabbits, we decided, for uniformity, to use the same dose to infect the guinea pigs, mice, and swine.

One pig and 5 animals of each abnormal host group were killed on each of 1, 2, 3, 4, 7, 9, 11 DAI.

III. Postmortem procedures

Unless noted otherwise, the following procedures were carried out on individual animals of each host species. Pigs, rabbits, and guinea pigs were stunned and mice were anesthetized, all were exsanguinated and eviscerated as soon as possible.

From 1 to 11 DAI, the liver, lungs, trachea, esophagus, stomach, and small intestine were removed from all animals. From 7 DAI, the cecum and colon were also removed from the mice, guinea pigs, and rabbits. Each organ was incised, washed, and soaked in 0.85% saline. The lungs and livers were comminuted in a Waring Blendor before soaking in saline. After the ingesta was collected, the organs of the digestive system were ground and soaked in saline and examined separately.

Examinations for larvae were made on the pooled washings of each organ-preparation from mice, guinea pigs, and rabbits; and, de-

Table 1. Distribution of larvae, identified to stage of development, recovered from swine, rabbits, guinea pigs, and mice killed 1 to 11 days after being experimentally infected with a single dose of 1,300 decoated embryonated eggs of *Ascaris suum*.

Host	Days after infection	Numbers of larvae recovered[1] Range (Average)	Total	Stage(s) of development attained;[2] and total numbers of each stage recovered from the following organ(s): Liver	Lungs	Small intestine
Swine	1	not applicable	60	L2; 60	0	0
	2	"	50	L2; 13 E3; 37	0	0
	3	"	65	E3; 65	0	0
	4	"	341	E3; 21 M3; 279	M3; 41	0
	7	"	624	M3; 2	L3; 622	0
	9	"	412	M3; 1	M3; 5 L3; 240	L3; 137 3M; 28 E4; 1
	11	"	434	0	L3; 100	L3; 5 3M; 83 E4; 247
Rabbit	1	0– 4 (1)[3]	6	L2; 6	0	0
	2	0– 20 (9)[4]	44	L2; 15 E3; 29	0	0
	3	2– 22 (15)	75	E3; 54	L2; 6 E3; 15	0
	4	10– 43 (23)	113	E3; 9 M3; 84	L2; 1 E3; 1 M3; 18	0
	7	98–236 (178)	890	M3; 2	E3; 55 M3; 273 L3; 550	0
	9	7–126 (79)	395	0	M3; 14 L3; 297	L3; 84
	11	40–115 (66)	329	0	E3; 1 M3; 9 L3; 31	L3; 27 3M; 44 E4; 216
Guinea pig	1	2– 24 (12)	60	L2; 60	0	0
	2	6– 34 (14)	70	L2; 26 E3; 44	0	0
	3	4– 46 (23)	115	L2; 15 E3; 58	L2; 6 E3; 36	0
	4	18–109 (51)	256	E3; 25 M3; 194	L2; 2 E3; 1 M3; 23	0
	7	31–150 (86)	429	0	M3; 171 L3; 258	0
	9	17– 89 (52)	259	0	E3; 3 M3; 40 L3; 213	L3; 3
	11	2– 88 (21)	104	0	E3; 4 M3; 31 L3; 69	0
Mice	1	8– 74 (34)	172	L2; 172	0	0
	2	2– 42 (23)	116	L2; 95 E3; 21	0	0
	3	145–326 (227)	1,137	L2; 138 E3; 999	0	0
	4	73–455 (262)	1,308	E3; 372 M3; 929	E3; 2 M3; 4	E3; 1
	7	4– 23 (16)	81	E3; 1 M3; 10 L3; 6	M3; 4 L3; 60	0
	9	0– 7 (2)[3]	11	M3; 2	M3; 1 L3; 8	0
	11	0– 15 (5)[4]	24	M3; 2	M3; 5 L3; 15	L3; 2

[1] Data from swine, based on 10% aliquots or total numbers of larvae recovered from 1 animal, on each day after infection.

For rabbits, guinea pigs, and mice: Data included under "range (average)," based on total numbers of larvae recovered from each member of a group of 5 animals, on each day after infection. The number given under *"total"* refers to the combined number of larvae recovered from each group.

[2] Stages: L2 = late second stage; E3, M3, and L3, respectively, early, middle, and late third stage; 3M = ensheathed larva in third molt; and E4 = early fourth stage.

[3] Two of 5 animals were negative for larvae.

[4] One of 5 animals was negative for larvae.

Table 2. Rate of growth[1] of *Ascaris suum* larval stages that developed in swine, rabbits, guinea pigs, and mice from a single dose of 1,300 eggs, up to 11 days after infection.

Stage of development[2]	Days after infection	Location	Total length and diameter at level of base of esophagus of larvae[1] from following: Swine	Rabbits	Guinea pigs	Mice
Late second	1 + 2	liver	180– 250 (236) × 12–17 (13)	194– 270 (219) × 12–16 (14)	206– 260 (241) × 12–14 (13)	180– 290 (237) × 12–17 (13)
Early third	2 + 3	liver	240– 460 (327) × 12–22 (18)	211– 450 (287) × 14–26 (17)	204– 330 (275) × 14–20 (17)	200– 400 (298) × 14–22 (17)
Middle third	4	liver	341– 650 (529) × 24–36 (33)	400– 750 (529) × 19–34 (25)	400– 620 (499) × 20–29 (23)	380– 690 (471) × 19–29 (25)
Late third	7	lung	980–1,830 (1,322) × 31–60 (45)	900–1,860 (1,497) × 36–75 (58)	970–1,670 (1,229) × 33–60 (46)	900–1,530 (1,225) × 30–70 (48)
	9 + 11	lung	1,000–2,000 (1,756) × 43–70 (60)	1,000–2,160 (1,615) × 42–80 (63)	950–1,800 (1,330) × 35–60 (52)	950–1,800 (1,480) × 38–65 (52)
	9	intestine	1,520–1,860 (1,716) × 50–60 (53)	1,600–2,080 (1,866) × 55–75 (65)	1,330–1,600 (1,450) × 50–60 (55)	none recovered
Third molt[3]	11	intestine	1,800–2,000 (1,938) × 50–60 (55)	1,750–2,000 (1,853) × 56–70 (60)	none recovered	none recovered
Fourth	11	intestine	1,810–2,850 (2,337) × 50–60 (55)	1,810–2,480 (2,080) × 50–80 (58)	none recovered	none recovered

[1] Ranges and (averages) in microns for 10 larvae, except for second stage and third molt from swine, which are based on respectively, 5 specimens; and for third molt from rabbits which are based on 3 specimens.
[2] Identifications of the stages are based on the descriptions given by Douvres et al. (1969). Larva in a molt denotes that it was completely enveloped by a sheath.
[3] Measurements for this stage, includes sheath in molting larvae.

pending on the numbers present, either pooled washings or 10% aliquots of washings of each organ-preparation from pigs. Larvae were put in saline and examined under magnifications of 30 and 60 diameters to estimate condition and viability. Larvae were then fixed in hot 5% formalin in Fenwick's (1939) balanced salt solution. When 50 or less larvae were recovered from an organ, all were studied. When more than 50 were recovered, 20 to 50% of the specimens were examined. Identification of stages and phases of development was based on the descriptions of Douvres et al. (1969).

Results

Larvae of *A. suum* were recovered from all swine and guinea pigs, 32 of 35 rabbits, and 32 of 35 mice (Tables 1 and 2). The 3 species of abnormal hosts were also infected with other parasites: *Passalurus ambiguus* and *Eimeria stiedae* in some rabbits; *Syphacia obvelata* in all mice; *Aspiculuris tetraptera* in some mice; *Hymenolepis nana* in the 5 mice killed on day 11; and *Paraspidodera uncinata* in some guinea pigs. Extraneous helminth parasites were not found in swine.

A. suum larvae recovered from the 4 hosts were anatomically normal and readily classifiable as follows: late second stage (L2); early (E3), middle (M3), and late third stage (L3), third molt (3M), and early fourth stage (E4). Larvae developed to the fourth stage in the normal host and rabbits; but only to beginning late third stage in guinea pigs and mice. No larvae in second molt were found in any host species.

I. Yields and migration patterns (Table 1)

Based on age of infection, the total numbers of larvae recovered from the 3 abnormal host species varied as follows: From 1 to 4 DAI, the numerical ranking was highest in mice, intermediate in guinea pigs, and lowest in rabbits. The numbers of larvae recovered on days 3 and 4, as compared with days 1 and 2, showed a 9 to 10 fold increase in mice and a 2 to 3 fold increase in guinea pigs and rabbits. The numbers of larvae recovered 7 DAI rose sharply in all host animals except mice; in these it fell to about 6% of the recovery at 4 DAI. From 7 to 11 DAI, the numerical ranking of larval recoveries was reversed, as follows: highest in rabbits, intermediate in guinea pigs, and lowest in mice.

The following describes the yields and advancement attained by the larvae recovered from the liver, lungs, and small intestine (Table 1) and data on larval stages recovered from

the cecum and colon, from the abnormal host animals.

A. Liver. All or most of the larvae recovered on days 1 to 4 were in the livers of all host species. Thereafter, a few were recovered from the livers of swine (7 and 9 DAI), rabbits (7 DAI), and mice (7, 9, and 11 DAI). L2, E3, and M3 larvae first appeared on days 1, 2, and 4, respectively, in all host species. M3 was the most advanced larval phase in the livers of all animals, except 2 mice that had L3 larvae on day 7.

From 1 to 4 DAI, the numbers of larvae in the livers were highest in mice, guinea pigs, and rabbits in that order (Table 1). Many L2 larvae persisted up to 3 DAI in mice (12%) and guinea pigs (21%); whereas, in rabbits and swine all larvae were E3 by 3 DAI. By 4 DAI, all larvae in the liver were E3 or M3. The ratio of phases 90% M3 to 10% E3 was essentially the same in all hosts except in mice where it was 71% M3 to 29% E3.

B. Lungs. Larvae migrated to the lungs in all abnormal hosts, being found at 3 DAI in rabbits and guinea pigs as a mixture of L2 and E3, and at 4 DAI in mice as a mixture of E3 and M3. In the normal host, larvae migrated to the lungs by 4 DAI when all found there were M3.

All or most of the larvae recovered on days 7, 9, and 11 from guinea pigs and mice were in the lungs; all or most of the larvae recovered on days 7 and 9 from swine and rabbits were in the lungs.

C. Small intestine. Larvae found in the small intestine of the 4 hosts were all in the ingesta. Substantial numbers of larvae were in the intestine of swine and rabbits as early as 9 DAI, but development through 3M and to E4 did not take place in rabbits until 11 DAI. All or most of the larvae recovered 11 DAI from swine and rabbits were in the small intestine. Although more larvae were recovered from swine than rabbits, virtually an identical percentage (74–75%) of E4 larvae was recovered from the two hosts (Table 1).

The 3 larvae recovered on day 9 from guinea pigs and the 2 larvae recovered 11 DAI from mice were no further advanced than beginning late third stage.

D. Cecum and colon. Examinations of the ingesta and tissues of the cecum and colon of mice, guinea pigs, and rabbits on 7, 9, and 11 DAI yielded one live L3 in one rabbit and 2 dead L3 and 2 live E4 in another at 11 DAI. The larvae were in the cecal ingesta of both rabbits.

II. Condition of larvae

Larvae retrieved from the saline preparations of the liver, lungs, and small intestine from swine, and with few exceptions, from the 3 other host species, were alive and normal. Dead larvae and those considered moribund because of degenerative changes, i.e., blistered cuticles or gut-less or vacuolated appearances, were recovered from the following animals: (1) on day 3, 2 mice with total counts of 149 and 230 larvae in their livers included 50% dead; (2) on day 3, 3 rabbits with total counts of 2, 5, and 12 larvae in their livers included 1, 1, and 2 specimens that were moribund; (3) on day 4, 1 rabbit with a total count of 13 larvae had 12 that were moribund in the liver and 1 dead in the lungs; (4) on day 7, 1 guinea pig with a total count of 44 larvae in the lungs had 4 that were moribund; and (5) on day 11, 1 guinea pig with a total count of 88 larvae in the lungs had 10 that were moribund.

III. Growth (Table 2)

Larval growth rates in the livers of all hosts were essentially similar through second and early third stage. However, in middle third stage at 4 DAI, the growth rate of guinea pigs and mice lagged behind that in rabbits and swine. This difference was more pronounced in the middle and late phases of third stage in the lungs at 7 and 9 DAI. From 9 to 11 DAI, larval growth rates in the intestine of rabbits and swine were essentially the same. Since few larvae underwent tracheal migration in guinea pigs and mice, no useful comparisons with these hosts can be made.

Discussion

The results obtained for the advancement, growth, and migration of *A. suum* to fourth stage in the normal host (swine) infected with 1,300 eggs were, with one notable exception, essentially identical to those previously reported (Douvres et al., 1969) in swine infected with 100,000 eggs. They found larvae in the second molt in the livers of swine between 18 and 36 hr. after infection; this phase was not found in

the present study. Comparisons herein between swine and the 3 abnormal hosts are based on the data from the present study.

Larval recoveries from all host groups increased during days 1–4, but among abnormal hosts were highest in mice and lowest in rabbits. It is unlikely that these increases could be due to varying infectivity of the eggs since the shorter period infections were done first. We consider the most probable explanation to be the accumulation of larvae in the liver. During days 1–4, when this buildup is taking place, larvae are reaching the liver from other body locations. Since we were concerned only with major sites, no attempt was made to account for this increment by examining the whole carcass. The fact that more larvae were recovered from mice than other abnormal hosts during this period might be attributed to superior recovery from a smaller volume of tissue. However, when these recoveries are compared with those from swine this argument becomes untenable. It would appear that other factors, including individual variation and fitness of the species as a host, are of greater importance.

As expected from our previous observations on early development in the rabbit (Douvres and Tromba, 1966 and Douvres et al., 1969), and the fact that *A. suum* reaches sexual maturity in this host (Berger et al., 1961), development in rabbits and swine up to 11 DAI was almost identical. As far as we know, there are no published reports in which the stages of development of *A. suum* larvae in rabbits are identified morphologically.

In mice and guinea pigs, as in the normal host, larvae begin third stage in the liver at 2 DAI and advance to beginning of L3 in the lungs at 7 DAI. However, no further development of the L3 occurs in the lungs of mice and guinea pigs. The occasional larva found in the intestine of the abnormal hosts is no further advanced than M3 or beginning L3. For the most part, migration and development in mice is limited to attainment of M3 in the liver, and in guinea pigs, they are limited to attainment of L3 in the lungs.

In view of the data reported herein, our previous conclusions (Douvres et al., 1969) relative to development of *A. suum* in normal versus abnormal hosts and the unreliability of identifications based on size, location in the host, or number of days of development are confirmed. However, if anatomical characterizations (Douvres et al., 1969) are used for identification, the early development in mice, guinea pigs, and particularly rabbits is sufficiently like that in swine to justify the use of these abnormal hosts as experimental models.

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Nematode Parasites of the Coelomic Cavity of Earthworms. X. A New Genus and Two New Species from New Guinea

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ABSTRACT: *Gatesnema bilobatum* g. n., sp. n. and *Iponema pheretimae* sp. n. from the earthworm *Pheretima bulmeri* (Gates, 1971) are described and figured. They belong to the Drilonematidae and were collected at an elevation of 8,300 feet in New Guinea.

Specimens of a new genus of nematode parasite of earthworms and a previously undescribed species of *Iponema* Timm and Maggenti, 1966 were received from Dr. G. E. Gates. Both species are from the same earthworm, a new species of *Pheretima* from New Guinea.

Genus *Gatesnema* gen. n.

Diagnosis: Drilonematidae. Head bearing two large lateral lobes extending anteriorly. Esophagus clavate. Anterior ovary with large elliptical spermatheca anterior to uterine–ovarian junction; hundreds of smooth-shell ova in uterus. Male with single reflexed testis and two equal spicules. Two large opposing circular phasmids on tail.

This genus is distinctive because of the lateral lobes of the head, which somewhat resemble the vesiculate amphids of the Desmoscolecida, although when the latter are lengthy they always extend backwards. It seems closest to *Tonoscolecinema* Timm, 1967 and *Burmanema* Timm, 1967.

Type species: *Gatesnema bilobatum* sp. n.

The genus is named in honor of Dr. Gordon E. Gates, earthworm specialist who for more than forty years has been saving the nematode parasites found in his dissections of earthworms.

Gatesnema bilobatum sp. n. (Fig. 1, A–G)

Holotype male: Length (L) = 4.51 mm; esophagus (e) = 0.25; posterior end of esophagus to anteriormost extension of testis (e–t) = 0.31; anteriormost extension of testis to anus (t–a) = 3.6; tail length (t) = 0.35; maximum body diameter (mbd) = 0.08.

Incomplete male (1): L = 3.36 mm; e = 0.29; e–t = 0.22; mbd = 0.1.

Females (4): L = 7.08–9.61 mm; e = 0.27–0.29; posterior end of esophagus to vulva (e–V) = 3.82–5.76; vulva to anus (V–a) = 1.90–2.90; t = 0.36–0.65; mbd = 0.14–0.19.

Description: Cuticle separated on all specimens, finely annulated, annules about 2 μ broad. Head bearing two large granular lateral

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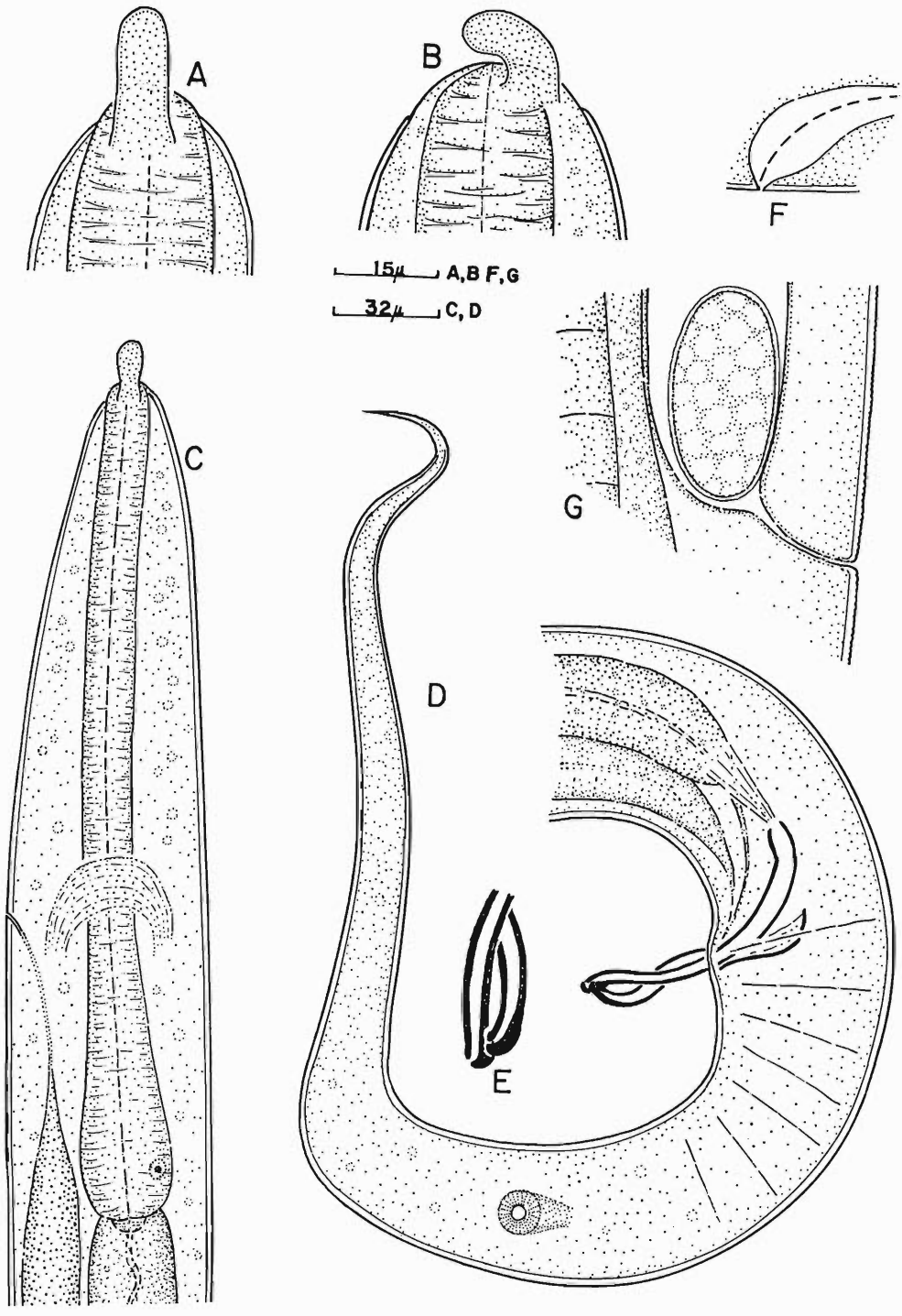
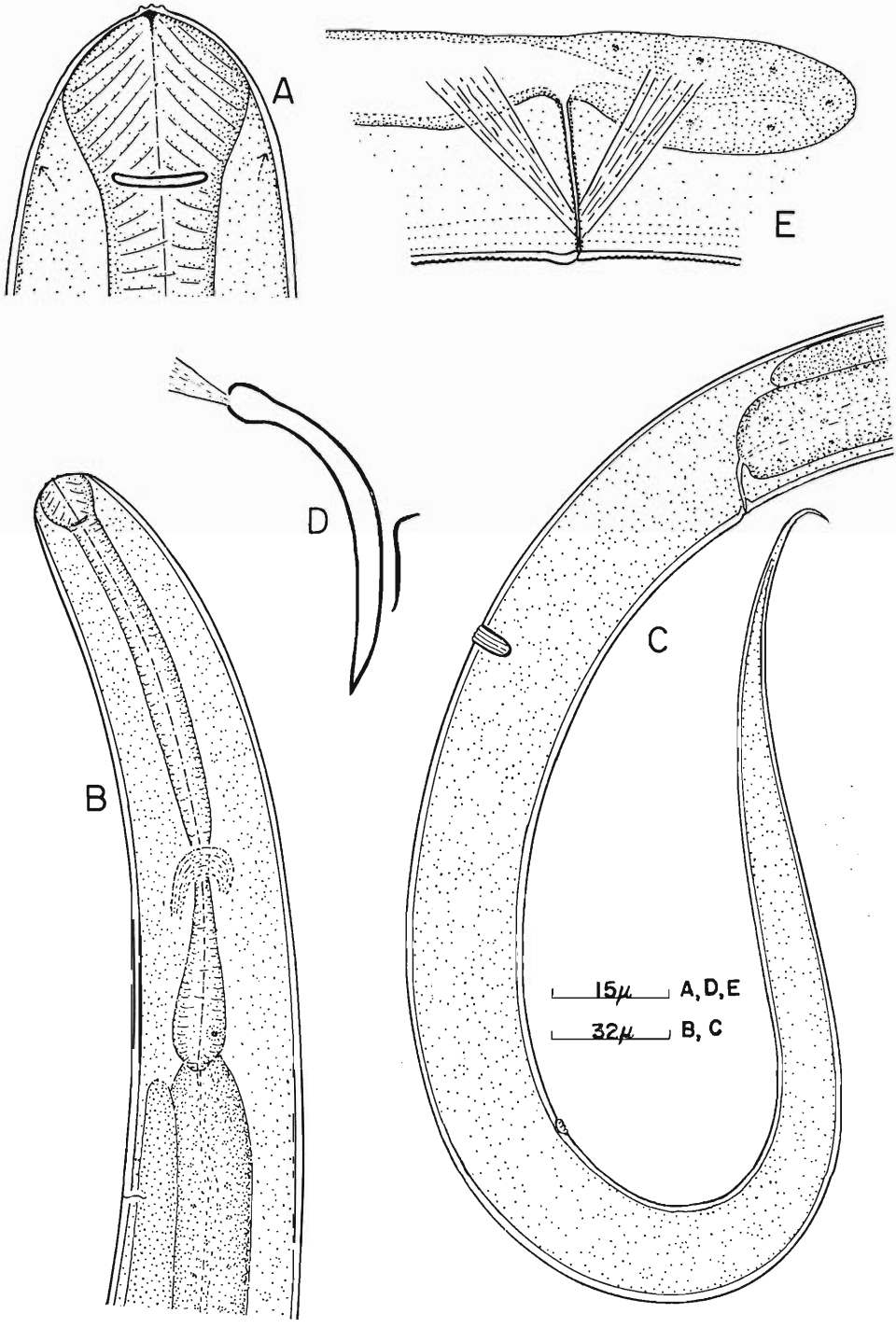
Figure 1. *Gatesnema bilobatum* gen. n., sp. n. A. Lateral view of female head. B. Ventrolateral view of female head. C. Esophageal region of female. D. Male tail. E. Tips of spicules. F. Phasmid of female tail, dorsal view. G. Vulvar region.





lobes protruding beyond head contour, either straight or curved (Fig. 1, A, B); papillae and amphids inconspicuous. Esophagus of uniform width up to slightly expanded base; nerve ring surrounding esophagus just anterior to swollen base (Fig. 1, C). Excretory pore inconspicuous, opposite nerve ring. Single anterior gonad. Vulva without lips; vagina inclined slightly anteriorly (Fig. 1, G). Broad uterus filled with hundreds of ova with clear shells, 51–61 μ × 25–27 μ; elliptical spermatheca, about 0.30 mm long, at junction of uterus and ovary. Ovary extending to anal region. Single testis in male, reflexed 0.15–0.16 mm at anterior. Two equal spicules with twisted tips, 90 μ long; gubernaculum 29 μ long. Tails in both sexes tapering uniformly to acute tip; tail 6.4 anal body diameters long in male, 6.6–8.8 in female. Faint circular phasmids at anterior fourth of tail, opposite each other, without conspicuous internal cavity.

TYPE HABITAT: Coelomic cavities of segments xi to xiii of the earthworm *Pheretima bulmeri* (Gates, 1971).

TYPE LOCALITY: Kai Ronk Valley, Schrader Range, New Guinea, elevation 8,300 feet.

HOLOTYPE MALE: Collected in August–September, 1968; deposited in U.S.D.A. Nematode Collection, Beltsville, Maryland, Cat. No. T-194t.

PARATYPES (4 females and anterior part of male): Same data as holotype; Cat. Nos. T-933p, T-934p, T-935p.

Iponema pheretimae sp. n. (Fig. 2, A–E)

HOLOTYPE MALE: L = 2.10 mm; e = 0.16; e–T = 0.41; T–a = 1.08; t = 0.37; mbd = 0.06.

OTHER MALES (4): L = 1.39–1.94 mm; e = 0.18–0.19; e–T = 0.27–0.35; T–a = 0.97–1.16; t = 0.31–0.39; mbd = 0.05–0.06.

FEMALES (10): L = 1.83–2.10 mm; e = 0.16–0.19; e–V = 0.64–0.84; V–a = 0.48–0.65; t = 0.42–0.48; mbd = 0.05–0.07.

DESCRIPTION: Cuticle thin, finely striated. Head broadly rounded, 29–32 μ in diameter, bearing four indistinct papillae; lips fused, oral lining slightly thickened. Amphids at level of cephalic papillae, elliptically flattened, with thickened rims (Fig. 2, A). Esophagus expanded in head region, with narrower isthmus and slightly swollen at base; nerve ring surrounding isthmus (Fig. 2, B). Terminal portion of excretory duct moderately cuticularized; excretory pore about one body diameter posterior to base of esophagus (Fig. 2, B). Ovary extending to anal region; large elliptical spermatheca anterior to junction of uterus and ovary. Vagina at right angle to body surface; short postvulvar uterine sac (Fig. 2, E). Ova few, 48 μ × 22 μ, without ornamentation. Testis single, reflexed slightly at anterior. Spicules about 40 μ long, cephalate; gubernaculum parallel, with a short posterior apophysis (Fig. 2, D). Tail in both sexes tapering uniformly to acute tip, 8–10 anal body diameters long in male, 12–14.5 in female. Caudal suckers elliptical, with fine transverse ribs between the dorsal and ventral margins; right sucker subdorsal, a short distance behind anus (48–80 μ); left sucker subventral, 130–144 μ posterior to right sucker (Fig. 2, C).

TYPE HABITAT: Coelomic cavities of segments xi to xiii of *Pheretima bulmeri* (Gates, 1971).

TYPE LOCALITY: Kai Ronk Valley, Schrader Range, New Guinea, elevation 8,300 feet.

HOLOTYPE MALE: Collected in August–September, 1968; deposited in U.S.D.A. Nematode Collection, Beltsville, Maryland, Cat. No. T-195t.

PARATYPE: (33 females and 4 males): Same data as holotype; Cat. Nos. T-936p to T-940p.

DISCUSSION: This species is closest in size to *Iponema minor* Timm and Maggenti, 1966, but the tail is longer and lacks a spicate tip in the male, the amphidial apertures are more slit-like and the caudal suckers are more widely separated from each other.

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Figure 2. ***Iponema pheretimae*** **sp. n. A. Male head. B. Esophageal region of female. C. Female tail. D. Copulatory apparatus. E. Vulvar region.**

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Research Note

Some Helminths of the Six-lined Lizard, *Cnemidophorus sexlineatus*, in South Dakota

Twenty-three *C. sexlineatus* collected in Todd County, South Dakota during the summer months of 1969 and 1970 were examined for helminths.

Thirteen lizards harbored *Oochoristica bivetellobata* Loewen, 1940 in the small intestine. Infections varied from 2 to 11 tapeworms with an average of 5.2 per host. The majority of parasites were found attached to the intestinal mucosa just posterior to the pylorus. Fully developed specimens were observed in only 9 lizards. This species has previously been reported from *C. sexlineatus* in Kansas (Loewen, 1940, Trans. Am. Microscop. Soc. 59: 511–518), *C. tigris* in Utah (Grundmann, 1959, J. Parasit. 45: 394), and Nevada (Babero and Matthias, 1967, Trans. Am. Microscop. Soc. 86: 173–177), and *C. hyperythrus* in California and Mexico (Bostic, 1965, Southwest Nat. 10: 313).

Tetrathyridia larvae of the tapeworm *Mesocestoides* were found in two lizards. A male contained 3 larvae located in the abdominal musculature and a female contained 10 tetrathyridia in the abdominal mesenteries as well as several free in the abdominal cavity. *Mesocestoides* larvae have previously been reported from *Sceloporus occidentalis* in California (Voge, 1953, Am. Midl. Nat. 49: 249–251; Specht and Voge, 1965, J. Parasit. 51: 268–272). Tetrathyridia have also been reported in other vertebrates, including frogs, toads, snakes, rodents, and carnivores.

Oxyurids, identified as *Pharyngodon werneri* Harwood, 1932 were observed in the cecum of 19 lizards. Two lizards also harbored specimens in the small intestine to which they probably migrated after the death of the host. This species has previously been reported from *C. sexlineatus* in Texas (Harwood, 1932, Proc. U. S. Nat. Mus. 81: 1–67) and *C. tigris* in Utah (Grundmann, *op. cit.*) and Nevada and Arizona (Babero and Matthias, *op. cit.*).

Immature specimens of *Physaloptera* were found in the stomachs of 7 lizards. Each lizard contained a single specimen with the exception of 1 which contained 3. Positive identification could not be ascertained. Babero and Matthias (*op. cit.*) found a single female specimen of *Physaloptera* in the stomach of *C. tigris* and identified it tentatively as *P. retusa* Rudolphi, 1819.

To my knowledge, there is no record of previous examination of *C. sexlineatus* for helminth parasites in South Dakota. The helminths mentioned in this report represent new locality records as *C. sexlineatus* parasites. The host, *C. sexlineatus*, represents a new host record for *Physaloptera* and *Mesocestoides*.

I am indebted to Dr. Richard Timken, Western Montana College, for field assistance and for confirming identification of lizards, and Mr. Peter Hillmann, Department of Zoology, Washington State University, for help in collecting hosts.

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Citellinema grisei sp. n. (Nematoda: Trichostrongylidae) from the Western Gray Squirrel, *Sciurus griseus*

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ABSTRACT: Two species of the nematode genus *Citellinema* are reported from the western gray squirrel, *Sciurus griseus*, collected in Oregon. This is the first report of helminth parasites from this host. One of the nematode species is described and named *C. grisei* sp. n.; the other is identified only as *Citellinema* sp. pending additional work on those species of the genus with spicules from 300 to 600 microns long. *C. grisei* is similar to *C. orientale* Schulz, 1933 and *C. nipponicum* Yamaguti, 1941 which have spicules from 750 to 1,100 microns long. *C. grisei* can be separated from these species by differences in: (1) distal ends of the spicules; (2) genital cone; (3) dorsal ray; (4) degree of asymmetry of bursa; and (5) ratio of lengths of anterior and posterior ovejectors.

Nematodes collected from the western gray squirrel, *Sciurus griseus* Ord, in Oregon were sent to the National Animal Parasite Laboratory for identification. They were collected between September 1965 and February 1966 by Stephen P. Cross, University of Arizona, Tucson. A search of the Index-Catalogue of Medical and Veterinary Zoology revealed no previous records of helminths from S. *griseus*. The western gray squirrel is distributed in parts of Washington, Oregon, California, and the extreme northern tip of the Baja peninsula in Mexico (Miller and Kellogg, 1955).

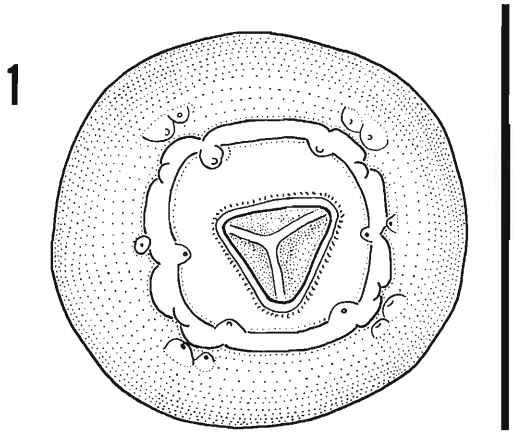
The nematodes were found to be: (1) an undescribed species of the genus *Citellinema* Hall, 1916 which is described below; and (2) from two of the four squirrels, specimens of another species of *Citellinema*, which cannot be determined specifically without further study of the species of the genus which have spicules 300 to 600 microns long.

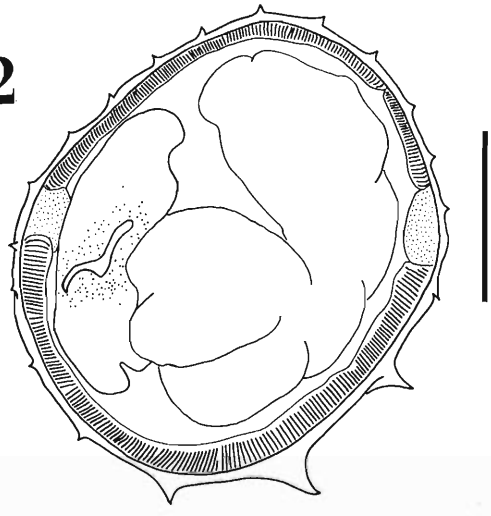
Description

All measurements are in microns unless otherwise stated.

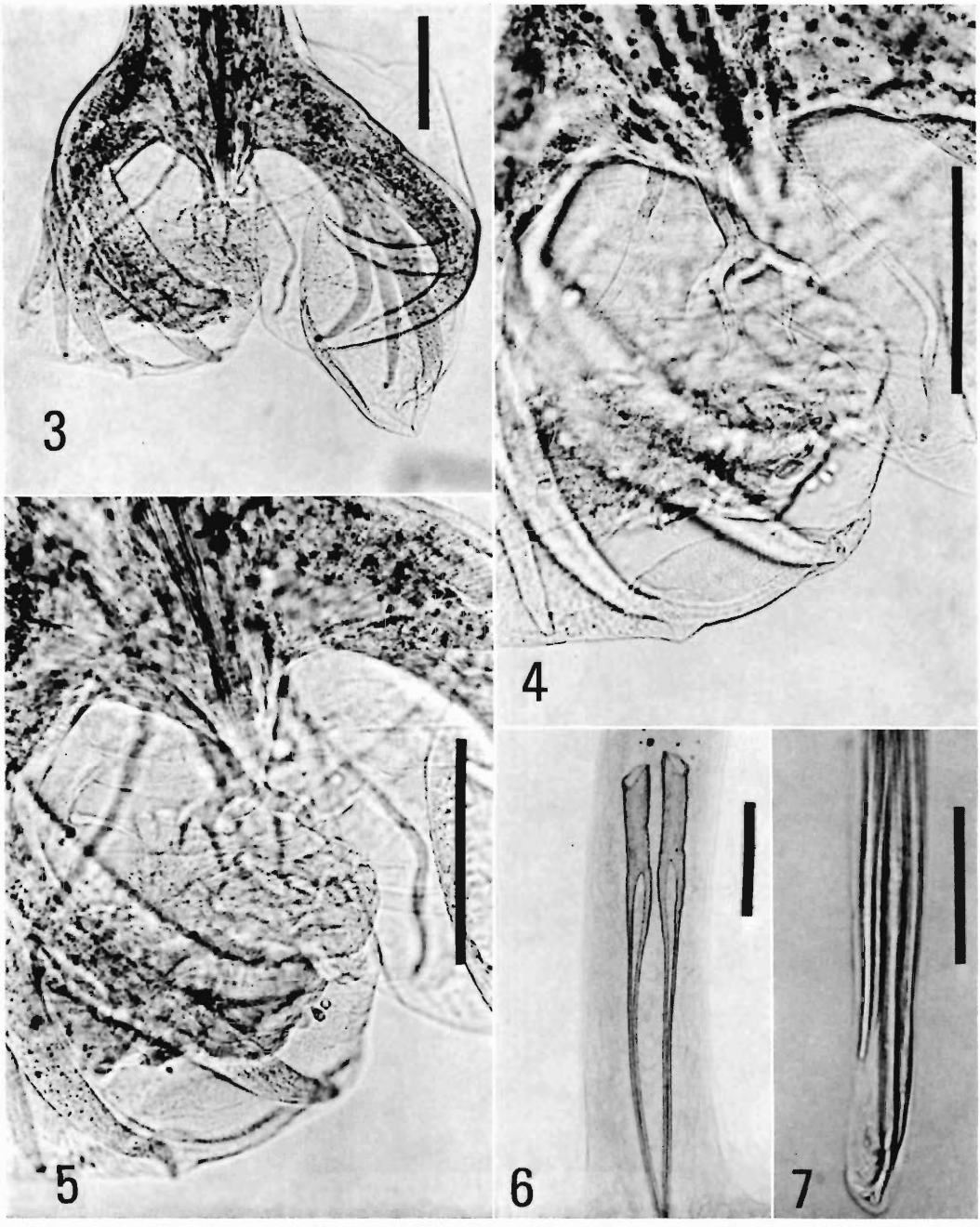
Citellinema grisei sp. n. (Figs. 1–11)

Long, slender, coiled nematodes; yellow in alcoholic preservative. Mouth triangular, bor-





Figures 1–2. *Citellinema griseus* sp. n. 1. *En face*. 2. Cross section through midbody of male. Scale bars 50 μ.



Figures 3–7. *Citellinema grisei* sp. n., Males. 3. Copulatory bursa, ventral view. 4. Dorsal and externodorsal rays of copulatory bursa, dorsal view. 5. Genital cone with lyre-shaped papillae and accessory bursal membrane, dorsal view. 6. Proximal ends of spicules, ventral view. 7. Distal ends of spicule, lateral view. Scale bars 100 μ in Figs. 3–6; 50 μ in Fig. 7.

dered by sclerotized rim; surrounded by six internal and eight external papillae (Fig. 1). Head with strongly annulated cephalic expansion (Fig. 8). Excretory pore near posterior end of esophagus, anterior or posterior to it.

MALE (Measurements of 12 nematodes from three squirrels): Length 8.6–14.1 mm. Width, at distal end of esophagus 75–105; prebursal 116–167. Esophagus 480–590 long. Cephalic expansion 90–159 long. Anterior end to: nerve ring 203–292; excretory pore 340–654. Seventeen longitudinal ridges on cuticle at anterior level of esophagus; 24 ridges just posterior to base of esophagus; most of anterior third of body bears 23–27 ridges; posterior ⅔ bears 19 ridges (Fig. 2). Prebursal papillae about 40 long. Bursa slightly asymmetrical, right lobe 335–482, left 308–442 (Fig. 3). Dorsal ray 76–108 long, bifurcated 54 from distal end, with short thin ramus laterally on each main branch slightly posterior to bifurcation (Fig. 4). Externodorsal and lateral rays characteristic of genus (Fig. 3). Genital cone elongate (sometimes as long as dorsal ray); bears two prominent lyre-shaped papillae that support accessory bursal membrane (Fig. 5). Spicules equal 831–1,060 long; each consists of cylindrical base 86–127 long and 19–28 in diameter, and two long slender tubular processes applied very closely together except for proximal region where prominent space occurs between processes (Fig. 6). One slender process of each spicule slightly shorter than other, ending in needle-like point that may be wavy; other process ending in blunt slightly expanded tip; both processes enclosed in membrane (Fig. 7).

FEMALE (Measurements of 12 nematodes from three squirrels): Length 15.8–25.2 mm. Width at distal end of esophagus 73–127; at anterior ovejector 146–194; at posterior ovejector 116–170. Esophagus 575–676 long. Cephalic expansion 100–140 long. Anterior end to: nerve ring 224–335; excretory pore 513–700. Thirty longitudinal cuticular ridges at distal region of anterior ⅛ of body; 24 ridges at distal end of anterior ⅖ of body; 22 ridges at midbody and posteriorly to level of posterior ovejector where there are 19 very small ridges. Vulva 3.24–5.22 mm from posterior end of nematode (Fig. 9). Anterior ovejector 1.00–1.36 mm long; posterior (Fig. 10) 724–971 long. Eggs oval 65–78 by 40–44. Tail 121–198 long. Tail spine 11–24 long (Fig. 11).

HOST: *Sciurus griseus* Ord, the western gray squirrel.

LOCATION: Small intestine.

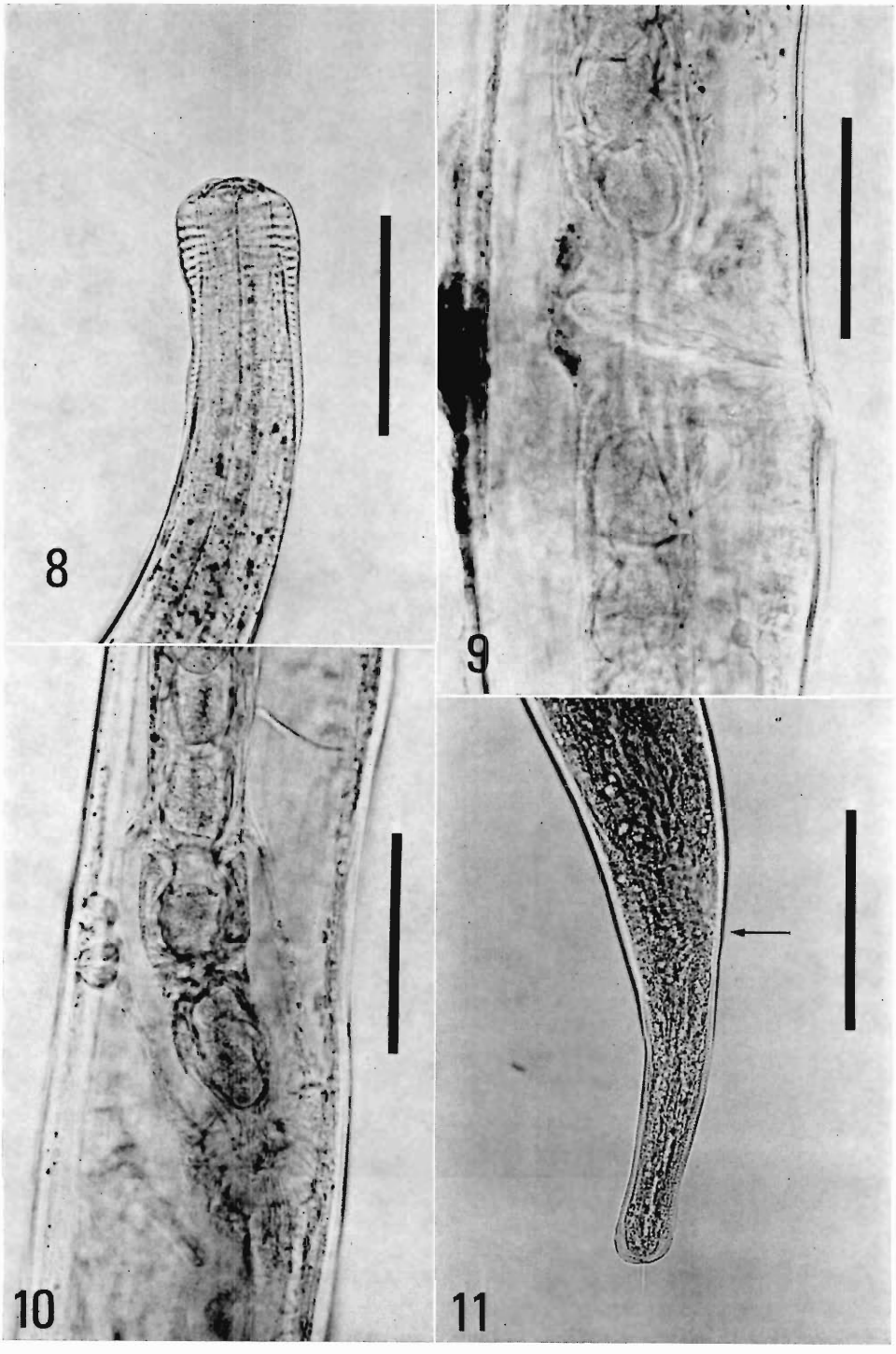
LOCALITY: Bald Mountain, Jackson County, Oregon, USA.

TYPE SPECIMENS: USNM Helm. Coll. No. 63206 holotype (male) and allotype; USDA Par. Coll. No. 66300.

Comparisons

All members of the genus *Citellinema* are parasitic in rodents of the family Sciuridae. Seven species have been previously described. However, Dikmans (1938) placed *C. monacis* Manter, 1930 and *C. sleggsi* Manter, 1930 in synonymy with the type species, *C. bifurcatum* Hall, 1916. Thus, most workers recognize five species: *C. bifurcatum*, *C. quadrivittati* (Hall, 1916), and *C. columbianum* Dikmans, 1938 from North America; *C. nipponicum* Yamaguti, 1941 from Japan; and *C. orientale* Schulz, 1933 from Siberia. The five species can be separated into three groups by the lengths of the spicules. *C. columbianum* spicules are very long (3.6 mm). Two species have spicules shorter than 700 microns; *C. bifurcatum*, including its synonyms, with spicules from 250 to 500 microns long and *C. quadrivittati* with spicules 695 microns long. Only *C. nipponicum* and *C. orientale* have spicules similar in length to those of *C. grisei*.

C. grisei is most similar to *C. orientale*, but differs from both that species and *C. nipponicum* in the following ways: (1) *C. grisei* spicules have an expanded blunt tip on the distal end of one of the tubular processes of each spicule. The lumen of the tubular process ends about 20 microns from the tip. In *C. orientale*, the tips are not expanded and the lumen ends less than 10 microns from the tip. *C. nipponicum* spicules have sharp tips. (2) The copulatory bursa of *C. grisei* is only slightly asymmetrical, but both Asian species have markedly asymmetrical bursae. (3) The dorsal ray of the bursa of *C. grisei* has much longer main branches than either Asian species. (4) The genital cone of male *C. grisei* is greatly elongated sometimes extending as far posteriorly as the distal tip of the dorsal ray. The genital cone of *C. orientale* is more blunt but does bear lyre-shaped papillae. According to Yamaguti (1941), the genital cone of *C. nipponicum* is



rounded without papillae. (5) In females, the difference in length of the anterior and posterior ovejectors of *C. grisei* is slight compared to that of *C. orientale*. The female of *C. nipponicum* is unknown.

Of the North American species, *C. grisei* is most similar to *C. quadrivittati* but can be separated from it by differences in spicule morphology. The proximal portions of *C. quadrivittati* spicules are conical rather than cylindrical and the tips are not expanded. These species also differ in the prominence of the genital cone and the degree of asymmetry of the bursa.

Remarks

The number of longitudinal cuticular ridges is one of the characters used by Skrjabin et al. (1954) to separate species of the genus *Citellinema*. Recently, Durette-Desset (1969) claimed differences in the number of cuticular ridges were sufficient to separate species that were made synonyms of *C. bifurcatum* by Dikmans (1938). However, the variation found in the number of cuticular ridges in different body regions of *C. grisei* emphasizes the need for an analysis of the variation of this character, both within and among specimens. Published descriptions of the number of cuticular ridges are often based on counts made on whole mounts with no study of variation. Longitudinal cuticular ridges can be studied in cross sections without time consuming histological procedures by cutting free-hand sections. This procedure is successful with small delicate heligmosomes as well as the larger trichostrongyles.

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Miller, G. S., and R. Kellogg. 1955. List of North American recent mammals. U.S. Natl. Mus. Bull. No. 205. 954 p.

Skrjabin, K. I., N. P. Shikhobalova, and R. E. S. Shul'ts. 1954. [Trichostrongylids of animals and man.] Osnovy nematodologii, Vol. 3. 683 p. (English translation, 1960. Washington, D.C.) (U.S. Dept. Commerce).

Yamaguti, S. 1941. Studies on the helminth fauna of Japan. Part 35. Mammalian nematodes, II. Japan J. Zool. 9: 409–439.

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Figures 8–11. ***Citellinema griseus*** **sp. n., Females. 8. Head and cephalic expansion. 9. Vulva. 10. Posterior ovejector containing two eggs with vestibule extending anteriorly. 11. Tail, anus (arrow), and tail spine. Scale bars 100 μ.**

Research Note

Helminth Parasites of the Cattle Egret in Puerto Rico

In a previous study of the helminth parasites of six species of birds in Puerto Rico (Whittaker et al., 1970, Proc. Helm. Soc. Wash. 37: 123–124), only five cattle egrets *Bubulcus ibis* (L.) were examined. To obtain additional information on the helminth fauna of this bird in Puerto Rico, 16 specimens of *B. ibis* were collected in May 1970 from the rookery near the University of Puerto Rico Biological Station at La Parguera and two specimens each near Isabella and Luquillo.

Table 1. Helminths found in 20 cattle egrets in Puerto Rico.

Helminth	No. cattle egrets infected	New record (*) of helminth for: Cattle egret	New record (*) of helminth for: Puerto Rico
Acanthocephala			
Centrorhynchus polymorphus Travassos, 1926 (cystacanth)	4	*	*
Nematoda			
Microtetrameres (Gynaecophila) egretes Rasheed, 1960	3	*	*
Desportesius invaginatus (Linstow, 1901) Skrjabin, Sobolev et Ivaschkin, 1965	1		*
Trematoda			
Prosthogonimus sp.	1	*	

The helminths found, the number of egrets infected with each parasite, and new host and locality records are listed in Table 1.

According to R. W. Macy of Portland State College, who examined stained specimens of the *Prosthogonimus* sp., the material does not appear to fit the description of any known species of the genus, and specific identification must await revision of the genus which he will soon undertake.

We are indebted to Mr. Vincent Resh for technical assistance. This study was supported by funds from the Arts and Sciences Research Committee of the University of Louisville.

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Research Note

Egg-Shell Precursors in Trematodes

There is evidence to indicate that a major portion of the trematode egg-shell is formed by the sclerotization of proteins, presumably from precursor substances, i.e., phenols, basic proteins, and phenol oxidase, found primarily in the vitellaria (Smyth and Clegg, 1959, Exp. Parasit. 8: 286–323; Smyth, 1966, The Physiology of Trematodes, Freeman, San Francisco; Clegg and Smyth, 1968 in Chem. Zool. Vol. II, Academic Press, N. Y.). The purpose of this report is to extend our knowledge of the occurrence of sclerotin egg-shell precursor substances in several trematodes.

Seven species of digenetic trematodes and one monogenetic trematode were studied. *Haematoloechus medioplexus*, *Megalodiscus*

Table 1. Histochemical tests for egg-shell precursors of sclerotin in several trematodes.

Trematodes	Phenols		Basic proteins		Polyphenol oxidase	
	Vitellaria	Ootype	Vitellaria	Ootype	Vitellaria	Ootype
Echinostoma revolutum	+++	+++	+++	+++	+++	+++
Echinoparyphium recurvatum	+++	+++	+++	+++	+++	+++
Haematoloechus medioplexus	+++	+++	+++	+++	+++	+++
Megalodiscus temperatus	—	—	++	++	—	—
Halipegus sp.	+++	++	+?	++	+	+
Gorgoderina sp.	+++	+++	+	+	—	—
Glypthelmins sp.	+++	+?	+	+?	+++	++
Polystomoides sp.	+++	++	+	+?	+++	++

+++ = very heavily positive.
++ = heavily positive.
+ = positive.
+? = questionable positive.
— = negative.

temperatus, *Halipegus* sp., *Gorgoderina* sp., and *Glypthelmins* sp. were obtained from naturally infected *Rana pipiens* frogs (Champlain Biological Co., Glen Gardner, New Jersey). The monogenetic trematode, *Polystomoides* sp. was obtained from naturally infected *Chrysemys picta belli* turtles (J. F. Schettle Frog Farm, Stillwater, Minn.). Two species of echinostomes, *Echinostoma revolutum* and *Echinoparyphium recurvatum* were reared experimentally in domestic chicks. Live worms obtained at necropsy and washed briefly in saline, were fixed and flattened between slides in warm 70% ethanol (Johri and Smith, 1956, Parasitology 46: 107–116). Most worms were fixed for a minimum of 24 hr and no longer than 1 wk prior to staining. Some specimens of *Megalodiscus temperatus* and *Gorgoderina* sp. were fixed for 2 hr (Saliternik and Clegg, 1967, cited in Clegg and Smyth, 1968 in Chem. Zool. Vol. II, Academic Press, N. Y.).

From 4 to 50 worms (aver. 15) were stained for each precursor substance; i.e., basic proteins, phenols, polyphenol oxidase. Basic proteins were identified with the malachite green technique (Smyth, 1951, Nature 168: 322–323; Johri and Smyth, 1956, loc. cit.), phenols with Fast Red Salt B (Johri and Smyth, 1956, Parasitology 46: 107–116), and polyphenol oxidase with the catechol technique (Smyth, 1954, Quart. J. Microscop. Sci. 95: 139–152). Whole mounts were prepared as described in the references cited except the worms' cuticles were punctured with insect pins following fixation. Preliminary work indicated that piercing of the cuticle facilitated infiltration of stains and provided uniform staining. Contrary to the findings of Johri and Smyth (1956, loc. cit.) no difficulty was experienced in preparing worms because malachite green stained whole mounts.

The results summarized in Table 1 reveal that basic proteins are present in the eight species, phenols in all but *M. temperatus* and the phenolase absent in *M. temperatus* and *Gorgoderina* sp. Histochemical identification of protein, phenol, and polyphenol oxidase in *H. medioplexus* confirms previous studies on frog lung flukes by Burton (1963, J. Exp. Zool. 154: 247–257) and Smyth (1954, loc. cit.). Positive reactions for the three precursors have been reported in *Polystomum integerrimum*, a species related to *Polystomoides* sp. by Kohlman (1961, Ztschr. Parasitenk. 20: 495–524). Guilford (1961, J. Parasit. 47: 757–764) reported the presence of protein and phenol oxidase in *Halipegus eccentricus*. The results of this study and those cited above suggest that *Echinostoma revolutum*, *Echinoparyphium recurvatum*, *Haematoloechus medioplexus*, *Glypthelmins* sp., *Halipegus* sp., and *Polystomoides* sp. utilize sclerotin in their egg shell capsules.

The absence of a polyphenol oxidase in *Gorgoderina* sp. confirms a previous study by Llewelyn (1965 in 3rd Symp. Brit. Soc. Parasit., Blackwell, London) on *Gorgodera vitelliloba* and *Gorgoderina* sp. by Johri and Smyth

(1956, loc. cit.). Absence of a polyphenol oxidase must be interpreted with caution as discussed by Read (1968 in Chem. Zool. Vol. II, Academic Press, N. Y.) since "the oxidation of catechol was used as the criterion for the enzyme; thus it can only be concluded that a catechol oxidase is absent from certain trematodes."

Negative results for phenol and polyphenol oxidase in *M. temperatus* confirm the observations of Madhavi (1966, Experientia 22: 93–94; 1968, Exp. Parasit. 23: 392–397) on two amphistosome species, *Diplodiscus meharai* and *Paramphistomum cervi*. He also showed the presence of large amounts of sulphated proteins in the two species, which may indicate that at least in some of the Paramphistomatidae keratin may be utilized in their egg-shell capsules.

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Research Note

A Redescription of *Anchoradiscus triangularis* (Summers, 1937) Mizelle, 1941 (Trematoda: Monogenea) from the Bluegill *Lepomis macrochirus* Rafinesque

The accessory plates on the dorsal and ventral bars of *Anchoradiscus triangularis* (Summers, 1937) Mizelle, 1941, were not mentioned in the generic description of *Anchoradiscus* Mizelle (1941, J. Parasit. 27: 159–163) but are present on both members of the genus. This species was first described by Summers (1937, J. Parasit. 23: 432–434).

Host specimens were collected by electric shocker during a study of the fish parasites conducted in Walter F. George Reservoir on the Chattachoochee River in Alabama. The hosts were placed in a 1:4,000 formalin solution as described by Putz and Hoffman (1963, J. Parasit. 49: 559–566) and after one hour formalin was added to make a 5% solution. Specimens were treated and measured as described by Mizelle and Klucka (1953, Am. Midland Naturalist 49: 720–733). Measurements are in microns; averages are followed by the range in parentheses. Illustrations were made with aid of a camera lucida.

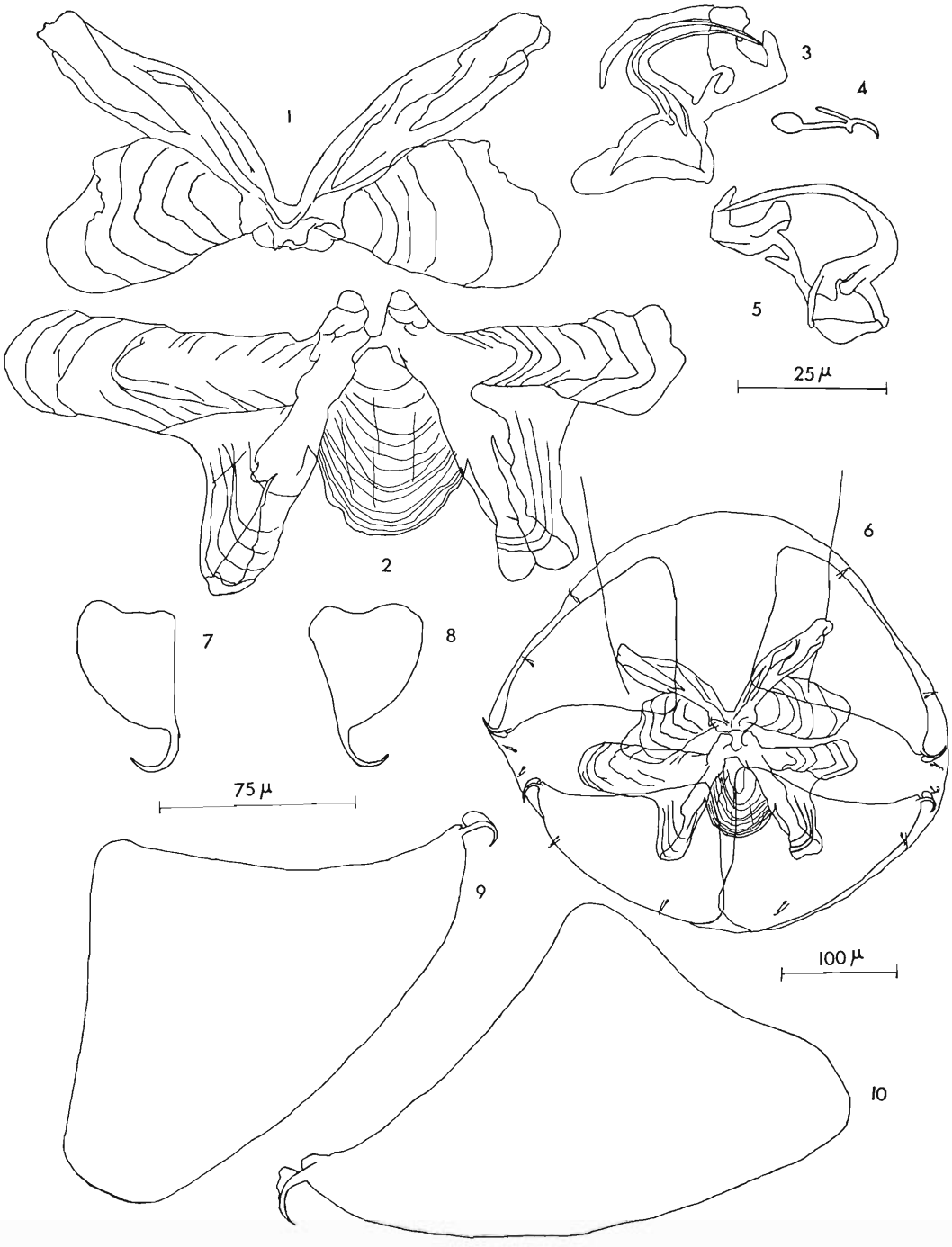
Anchoradiscus triangularis (Summers, 1937) Mizelle, 1941

Redescription

Dactylogyridae, Ancyrocephalinae: Length 561 (470–760), width 160 (120–250). Well defined head organs in groups of four on either side of convex cephalic region. Granular eyespots four, anterior pair smaller, farther apart. Pharynx circular to ovate, transverse diameter 39 (28–60). Haptor discoidal (Fig. 6), 211 (130–350) by 246 (170–390), joined to body by stout peduncle. Anchors large, base apparently expanded into triangular concave plates of similar shape. Ventral anchors (Fig. 10)

→

Figures 1–10. ***Anchoradiscus triangularis*** **from the bluegill. 1. Ventral bar. 2. Dorsal bar. 3. Mature copulatory complex. 4. Hook. 5. Immature copulatory complex. 6. Haptor. 7. Immature dorsal anchor. 8. Immature ventral anchor. 9. Dorsal anchor. 10. Ventral anchor.**



slightly larger, 142 (94–222) by 74 (60–128). Dorsal anchors (Fig. 8) 129 (88–198) by 61 (50–114). Wings conspicuous on anchor shaft. Bars articulated by two pairs of knobs near midpoint. Ventral bar (Fig. 1) consists of two well sclerotized arms, 74 (55–100) by 17 (13–21), distance between distal arm tips 112 (86–169), joining at articulation of bars; and two lamellar lateral accessory plates, 42 (28–86) by 29 (21–38). Dorsal bar (Fig. 2) consists of two well sclerotized arms, 73 (53–94) by 13 (8–19), and two lamellar lateral accessory plates, length 41 (65–108). Hooks (Fig. 4), length 16 (12–18), with posteriorly projecting appendage reaching distally inflated base. Cirrus (Fig. 3) a sickle-shaped tube expanding basally and articulating with accessory piece, length 28 (23–34). Accessory complex composed of a bifurcate accessory piece with protuberance on basal ramus and a wedge shaped portion of cirrus sheath articulating with ramus terminations, length 27 (21–36). Vagina a tapering tube terminating dextromarginally within sclerotized umbrellalike portion of integument, length 28 (26–30). Ovary ovate; large seminal receptacle ellipsoid; vitellaria diffuse; testis, seminal vesicle, and prostates not observed; intestinal crura confluent posteriorly.

Remarks

The sclerotized portion of the cirrus sheath (Fig. 5) may be inconspicuous or absent in the immature or young adult forms. Immature specimens also have a disproportionately large anchor point (Fig. 6) which attains adult size early in development of the anchor.

This is the first report of the species from the bluegill, *Lepomis macrochirus* Rafinesque, although Allison and Rogers (1970, Proc. Helm. Soc. Wash. 37: 17–23) reported the genus on bluegills in Alabama.

The authors would like to thank Dr. J. D. Mizelle for his comments on the species and the several workers who aided in collection of the specimens.

HOST AND LOCALITY: Bluegill, *Lepomis macrochirus* Rafinesque, Walter F. George Reservoir, Russell County, Alabama.

PREVIOUSLY REPORTED HOST AND LOCALITY: Bantam Sunfish, *Lepomis symmetricus* Forbes, Baton Rouge, Louisiana.

SPECIMENS STUDIED: 776 (11 measured).

TYPE SPECIMENS: Hypotypes, USNM Helm. Coll. No. 71807.

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Research Note

Nonrelationship Between the Time of Day when Guinea Pigs are Inoculated with *Trichostrongylus colubriformis* (Nematoda) and the Number of Worms Established

Many parameters of physiologic functions of animals fluctuate according to regular circadian periodicities. There is some evidence that mice are more resistant to injections of endotoxins injected at night when adrenal activity is increased (Halberg and Stevens, 1958, Fed. Proc. 17: 439). To determine whether the time of day at which guinea pigs were inoculated with the ruminant parasite, *Trichostrongylus colubriformis*, might affect the number of worms ultimately established, two tests were conducted.

In one trial, 10 female 10-week-old guinea pigs were each inoculated orally with 5,000 ± 202 infective larvae of *T. colubriformis* (RLS isolate) at 0900 hr and 10 others at 2100 hr. A second trial comprised 8 groups of 10 guinea

pigs each, and inoculations of 5,000 ± 229 larvae were made at 3-hr intervals between 0230 and 2330 hr. Groups were killed 8 days after inoculation, at the same time of day as originally inoculated. The entire small intestine of each guinea pig was removed and exposed to a 1% pepsin-HCl acid solution for digestion at 40 C for 4 hr. Numbers of worms were determined on the basis of counts of duplicate 3% aliquots per guinea pig.

The minimum, maximum, and mean numbers of worms per group ranged, respectively, from 970 to 1,845, 2,960 to 4,150, and 1,565 to 2,562. Analysis of the differences by Student's "t" in the first trial and analysis of variance in the second, indicated clearly that the time-of-day of inoculation does not influence the number of worms that will ultimately become established in the host.

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Research Note

Sclerotinoid cirrus in *Diplectanum lacustris* Thurston and Paperna, 1969 (Monogenea, Diplectanidae)

In the original description of *Diplectanum lacustris* by Thurston and Paperna (1969, Proc. Helm. Soc. Wash. 36: 214–218) the copulatory organ was reported as lacking any sclerotization or accessory piece usually found in other members of this genus. Additional material collected from the same hosts (*Lates niloticus* (L.), Ghana; *L. albertianus* Worthington, Uganda) was mounted unstained in Polyvinyl-Lactophenol and also in Glycerine jelly. In these specimens a delicate sclerotized structure, the cirrus, could be seen at the distal end of the vas deferens (Figs. 1, 2). The cirrus, 55–60 μ long, 16–25 μ wide, is spatula shaped, with its lateral margins folded inwards it produces a tube with a ventral slit, an additional V-shaped structure (accessory piece) is attached to the ventral side. The cirrus of this species differs distinctly from that of *D. latesi* described from *Lates calcarifer* Bloch from India, the only other known species of *Diplectanum* from fish of the genus *Lates* Cuvier. The two species also differ from each other in the structure of the anchors and the bars.

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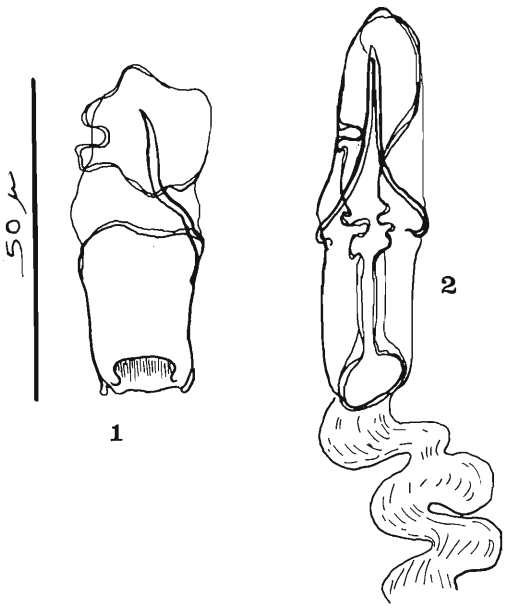

Figures 1, 2. Copulatory organ of *Diplectanum lacustris*, lateral (1) and ventral (2) view.

Research Note

Helminth Parasites of the Black-billed Magpie, *Pica pica hudsonia*, in Northeastern Colorado

In order to determine the parasite burden of the Black-billed magpie, *Pica pica hudsonia* (Sabine, 1823), in northeastern Colorado, 30 magpies were collected by shotgun in the vicinity of Greeley, Colorado and examined for helminths. Each bird was necropsied within 48 hours after collection. Cestodes were fixed in alcohol–formalin–acetic acid (AFA) solution, stained with aceto-carmine, and mounted in piccolyte; nematodes were fixed in AFA solution and mounted in glycerine or glycerine jelly. All birds were collected between September, 1965 and June, 1966. The results of our survey, presented in Table 1, include the percent of birds infected for each parasite and 5 new geographic records for Colorado.

Table 1. Helminth parasites of 30 magpies in Colorado.

Parasites found	% of birds infected	New record for Colorado
Cestodes		
Anomotaenia constricta (Molin, 1858)	16.5	*
Hymenolepis farciminosa (Goeze, 1782)	26.4	
Hymenolepis stylosa (Rudolphi, 1809)	16.5	*
Nematodes		
Acuaria anthuris (Rudolphi, 1819)	36.4	*
Capillaria corvorum (Rudolphi, 1819)	16.5	*
Microtetrameres corax Schell, 1953	62.9	*
Splendidofilaria caperata Hibler, 1964	3.3	
Splendidofilaria picacardina Hibler, 1964	26.4	

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MINUTES

Four Hundred Fifty-third Through Four Hundred Sixtieth Meetings

453rd Meeting: Naval Medical Research Institute, Naval Medical Center, Bethesda, Maryland, 16 October 1970. Dr. L. S. Diamond presented the Society's Anniversary Award to Dr. A. O. Foster and gave a biographical sketch of Dr. Foster's outstanding career. Papers presented: "Summary of a four-year epidemiological study on the blood parasites of a population of English sparrows," by J. Applegate and J. A. D'Adamo; "Stimulatory effect of skin lipid fractions on cercarial penetration," F. Austin, M. Stirewalt, and R. Danziger; "Fine structure of the exoerythrocytic stages of *Plasmodium lophuriae*," R. Beaudoin, C. P. A. Strome, and F. Mitchell; "Activities at NAMRU-3 in Ethiopia," J. Armstrong.

454th Meeting: National Animal Parasite Laboratory (Beltsville Parasitological Laboratory), Beltsville, Maryland, 20 November 1970. Slate of officers for 1971 presented: E. J. L. Soulsby (President), F. W. Douvres (Vice President), T. K. Sawyer (Recording Secretary), E. M. Buhrer (Corresponding Secretary-Treasurer). These were approved unanimously. Papers presented: "Immunization of cattle against *Oesophagostomum radiatum*," H. Herlich, F. W. Douvres, and R. D. Romanowski; "The structure and possible function of coelomocytes of nematodes," M. B. Chitwood and P. A. Madden; "Quinine inhibition of host cell penetration by eimerian sporozoites in vitro," R. Fayer.

455th Meeting: Patuxent Wildlife Research Center, Laurel, Maryland, 11 December 1970. Newly elected officers were installed. Papers presented: "Whirling disease of trout and salmon; a global problem," Glenn L. Hoffman; "Parasitological Research at Memorial University, St. John's Newfoundland," Carlton M. Herman; "New data on the biology of *Simulium innocens*," I. B. Tarshis; "Taxonomic criteria for the identification of amoebae of marine and freshwater fish," T. K. Sawyer, Glenn L. Hoffman, and John G. Hnath.

456th Meeting: National Institutes of Health, Bethesda, Maryland, 15 January 1971. Papers presented: "Antibody and immunoglobulin responses in malaria," John F. Finerty and Charles B. Evans; "The interaction of *Trypanosoma cruzi* with mouse peritoneal macrophages," James A. Dvorak and Gabriel A. Schmunis; "An epidemiologic approach to Chagas' Disease in Nicaragua," Franklin A. Neva; "Role of nonheme iron in cestode respiration," Eugene C. Weinbach.

457th Meeting: Walter Reed Army Institute of Research, Washington, D. C., 19 February 1971. Papers presented: "The effect of antilymphocyte and antimacrophage serum in *P. berghei* infections," Seth H. Lourie; "Biosynthesis of trehalose in *Moniliformis dubius*," Robert O. McAlister; "Intestinal helminthiases in rural Georgia," Larry K. Martin; "Laboratory colonization of tsetse flies," Ronald A. Ward; "WRAIR parasitological activities in Thailand," Carter L. Diggs.

458th Meeting: U. S. Department of Agriculture, Beltsville, Maryland, 19 March 1971. The treasurer's report and Auditing Committee's report was presented and accepted by the membership. Papers presented by University of Maryland: "Faculatative Parasitism," John O. Corliss; "Taxonomy of Microsporidia," Victor Sprague; "Rumen Microbial Influences on Ruminant Lipids," Mark Kenney; "Lipids of *Turbatrix aceti*," Lorin W. Krusberg; "A Few Post-Congress Notes," Gilbert F. Otto.

459th Meeting: The Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Maryland, 23 April 1971. Papers presented: "Competition between larvae of *Aedes* (*Stegomyia*) *albopictus* Skuse and *Aedes* (*S.*) *polynesiensis* Marks," Robert Lowrie, Jr.; "The effects of temperature on the development of cysticercoids of *Hymenolepis diminuta*," Stanton Parmeter; "Preliminary observations on the development of the gut of *Schistosoma mansoni*," Robert Rew; "Cultivation of *Leishmania donovani* at mammalian body temperature in a cell free medium," Bruce Weiss.

460th Meeting: Alumni House, New Bolton

Center, Kennett Square, Pennsylvania, 15 May 1971. Papers presented: "Pathological and immunological correlations in fascioliasis," Terence J. Hayes; "Granuloma formation to the egg of *Capillaria hepatica*: Further studies," Gene B. Solomon; "Cell mediated immunity responses in cutaneous leishmaniasis of the guinea pig: Further studies," Theodosia M. Welch; "Peripheral blood lymphoid cell responses in haemonchosis," Priscilla Chen; "Experimental toxocariasis," Stanislus T. Fernando; "Techniques used in experimental ascariasis," E. J. L. Soulsby. Cocktails were served in the Allam House, after which members and guests enjoyed dinner in the Alumni House.

The following were elected to membership at the meetings indicated: *453rd*: W. R. Anderson, R. A. Campbell, Dudley St. A. Chin, L. C. Gasbarre, O. T. Mehre, R. M. Reidel, R. A. Sanchez-Beaujon, F. M. Seesee. *454th*: B. E. Beacham, B. W. Erickson, Jr., D. B. Pence, E. L. Suydam. *456th*: K. de Soyza, G. P. Jaiswal. *457th*: W. H. Leigh, R. S. Wacha, S. D. Kalyankar, D. Matthias, K. A. Walker. *458th*: A. S. Murty, G. T. Fincher, A. L. Kocan, M. Vilchez. *459th*: C. Lee, S. Lloyd. *460th*: D. Ellington.

THOMAS K. SAWYER
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