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SUMMARY

The method of McCoy for obtaining hookworm eggs free from feces and growing the larvae in pure cultures of bacteria presented a means of comparing the viability and rate of development of the eggs and larvae of the two physiological strains of *Ancylostoma caninum*. For eggs of the dog strain, there was obtained a percentage hatching of 89 per cent at 21°C. and 50 per cent at 31°C. as compared to 28.5 per cent and 20 per cent respectively at similar temperatures for the eggs of the cat strain. Studies of the development of the eggs to the infective larval stage showed, for the dog strain, 44 per cent at 21°C. and 47 per cent at 31°C., and for the cat strain, 5 per cent at 21°C. and 6 per cent at 31°C. From these figures the percentage development of hatched larvae to the infective larval stage was computed. For larvae of the dog strain, this figure was 49 per cent at 21°C., and 94 per cent at 31°C., while for the cat strain, the figures were 17 per cent and 30 per cent respectively. These findings add to the number of experimentally demonstrated differences between the cat and dog strains of *A. caninum*.

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# STUDIES ON CILIATES FROM BERMUDA SEA URCHINS*

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WITH INTRODUCTION AND NOTES BY

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## INTRODUCTION

Available records indicate that Hoffman (1871) was the first to call attention to the existence of ciliates in the digestive tract of European sea urchins, but he gave no descriptions. Maupas described *Cryptochilum echini* from the Mediterranean urchin, *Strongylocentrotus* (*Echinus*) *lividus* in 1883, and since then additions have been made by Di Mauro (1904), Andre (1910), Russo (1914) and Hentschel (1924).

It appears that Jacobs (1914) was the first to record the presence of ciliates in American sea urchins. He mentions four kinds which he found in *Diadema setosum* and studied physiologically at the Tortugas Islands, but he gives no names, referring to them by the letters, A, B, C, and D. Bray (1925) observed ciliates in *Toxopneustes variegatus* at Bermuda in 1919 and in the same host at Beaufort, N. C., in 1924.

In the summer of 1923 Miss Ruth Jane Ball (now Mrs. Walter T. Biggar), then assistant professor of zoology at the University of Vermont, made a study of the ciliates of the sea urchins of Bermuda. Mrs. Biggar presented an account of her studies at the Washington meeting of the American Society of Zoologists, in December, 1924, suggesting new names for five species. In the abstract (Ball, 1924) which was published, however, no new names were included. Subsequent preoccupation has prevented Mrs. Biggar from adding to her studies or preparing her material for publication.

Meanwhile, Dr. J. E. Lynch has begun an investigation of the ciliates of the sea urchins of the Pacific coast and has already published two papers (Lynch, 1929, 1930). In 1930, further studies were begun on the ciliates of the Bermuda sea urchins by Dr. Miriam Scott Lucas, and on those from the urchins of the coast of Maine by Mr. Philip Powers.

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* Contributions from the Bermuda Biological Station for Research. No. 166.

At the request of Dr. E. L. Mark, Director of the Bermuda Biological Station, the writer has undertaken to prepare for publication a short paper giving the main results obtained by Mrs. Biggar. In the preparation of this paper the writer wishes to acknowledge, with thanks, the advantages he has had of personal conferences and correspondence with Dr. Lucas, Dr. Lynch and Mr. Powers, and an opportunity to examine some of their material. With these aids, and especially with the help of slides prepared by Dr. Lucas, and material sent from Bermuda by Mr. J. Kenneth Donahue, an attempt has been made to give a taxonomic status to four of the ciliates, the drawings and descriptions of which are found in Mrs. Biggar's notes. The fifth species mentioned by Mrs. Biggar was not characterized in sufficient detail to be named.

In arranging Mrs. Biggar's material, her original drawings have been used together with her descriptive notes. It should be mentioned that Mrs. Biggar's studies were limited largely to the living animals, hence some details are insufficiently indicated in the drawings. Likewise, her measurements are somewhat different from those obtained from preserved animals. In her notes, Mrs. Biggar assigned all four species to new genera, but since she did not mention any names in her published abstract, the writer has found it advisable to assign the four new species to existing genera. However, Mrs. Biggar's new species names are used. The notes and discussions added by the writer are enclosed in brackets. [D. H. W.]

## DESCRIPTION OF SPECIES

### Genus METOPUS Claparède et Lachmann 1858-59

### *Metopus circumlabens* n. sp. (Figs. 1 and 1a)

Morphology: Elongate-oval in outline, rounded anteriorly and tapering posteriorly in such a manner as to suggest an interrogation mark; region anterior to oral groove hook-like and cristiform; body flattened, especially anteriorly; animal highly flexible, particularly the anterior hook-like flap or crest. Peristome a deep furrow extending from a notch in the upper left hand margin (right in Fig. 1) in a sweeping curve parallel to the anterior border, narrowing into a short pharynx near the right margin and near the transverse axis; numerous food vacuoles confined to the posterior part of the body; anal opening at posterior extremity (Fig. 1a; egestion shown in Fig. 1). Cilia distributed over the body in oblique rows, somewhat longer anteriorly, especially differentiated along the lower border of the peristome [zone of membranelles]; a tuft of about twelve much longer cilia at the posterior end; a flagellum [undulating membrane?] protruding from the lower border of the pharynx. Three contractile

vacuoles, one near the posterior end, one to the right of the gullet and the third opposite the second on the left side of the animal. Macronucleus oval in profile, immediately posterior to the peristome [accompanied by a single micronucleus]. Size: 170 by 100$\mu$. Swims usually in a circular path.

Hosts: Found in large numbers in *Diadema setosum* and in *Echinometris subangularis*. Corresponds to species "B" of Jacobs.

[Mrs. Biggar had placed this new species in a new genus, but its obvious relationships to the species of Metopus, as recently defined by Kahl (1927), make it seem more logical to place it in the latter genus.]

Genus CRYPTOCHILUM Maupas 1883

*Cryptochilum bermudensis* n. sp. (Fig. 2)

Morphology: Shaped like a scoop; anterior two-thirds of body thin and leaf-like; posterior region thickened, terminating in a rudder-like style. Protoplasm colorless, transparent, highly alveolar in structure. Oral aperture on left border [as drawn] in the posterior third of the animal; food vacuoles confined to the posterior third of the body; anal aperture not observed. Cilia arranged in rows, indicated by 35 to 40 longitudinal striations on a side; more conspicuous on anterior margin, where they appear to be inserted on conical elevations; also more noticeable in the region of the cytostome; a group of longer cilia on the caudal style. Contractile vacuole single, surrounded by several small vacuoles, located near the posterior end. Macronucleus single, rounded, near the middle of the body; micronucleus single, close to the macronucleus, often containing three refractive granules. Size 130 by 75$\mu$. Movement very moderate as to speed, often in a circular path, rotating on its longitudinal axis.

Reproduction: Several seen in transverse fission.

Hosts: Abundant in all of 50 specimens of *Toxopneustes variegatus* examined. Probably corresponds to species "D" of Jacobs.

*Cryptochilum echinometris* n. sp. (Figs. 3 and 4)

Morphology: Has scoop-like form as in the preceding species, but is smaller, relatively narrower, with anterior end more symmetrically rounded, and has fewer longitudinal striations. Protoplasm transparent but in the anterior region densely granular instead of alveolar. Oral aperture farther forward than in *C. bermudensis,* but posterior to the middle of the body; food vacuoles confined to posterior region. Contractile vacuole single, near posterior end. Rounded macronucleus near center of body accompanied by one micronucleus. Size 73 by 26$\mu$.

Host: Found only in *Echinometris subangularis.*

[The two preceding species were assigned by Mrs. Biggar to a new genus. However, they appear to have such close affinities to *Cryptochilum echini* Maupas that they are now assigned to the same genus. Of the two, *C. echinometris* is the more nearly related to *C. echini,* but it has a somewhat different contour and the cytostome is considerably behind the middle of the body, whereas that of *C. echini* is at, or slightly anterior to, the middle. The nucleus of *C. echinometris* is also located more posteriorly. *C. bermudensis* (Fig. 2) is still further removed from the type species, showing an increase in size and in relative width, and having a still more posterior position for the cytostome. That these species are related to *Cryptochilum echini* is indicated by the report of Bray (1925), who states that he found *"Cryptochilum echini"* in all but one of 22 specimens of *Toxopneustes variegatus* secured at Beaufort, N. C., and that he saw a form resembling *C. echini,* but possibly a new species, in the same urchin collected in Bermuda.]

Genus ANOPHRYS Cohn 1866

*Anophrys elongata* n. sp. (Figs. 5 and 6)

Morphology: Body cigar-shaped, anterior end somewhat pointed, posterior end more rounded, anterior third narrower, longitudinally striated, very transparent and flexible (Fig. 5); posterior two-thirds contains many refractive bodies. Mouth obscure, ingestion not observed; anal aperture not determined. Cilia in longitudinal rows, more evident in the anterior third of body; a ciliated membrane extends out from the anterior third as far back as the oral indentation [not shown in drawings]. Contractile vacuole single, located close to the posterior extremity. Macronucleus near the center of the body. [A micronucleus close beside it.] Size: 166 by 33$\mu$. Movement very rapid, darting through the water in a straight or spiral path.

Reproduction: Several specimens seen in transverse fission.

Hosts: *Toxopneustes variegatus* and *Echinometris subangularis.* Probably corresponds to species "C" of Jacobs.

[The description and drawings of this ciliate as recorded by Mrs. Biggar are somewhat incomplete. Specimens on a slide prepared by Dr. Lucas show that the cilia are quite long, arranged in about 16 to 18 longitudinal rows and that there is a long caudal cilium or filament in addition. The oral membrane mentioned by Mrs. Biggar is not shown on her drawings, but appears to extend from the anterior end to the oral vestibule and along the right side of this cavity. It appears to consist of long cilia, set close together, gradually increasing in length backward to the oral depression and somewhat shorter within that cavity. In life they would probably simulate a membrane.

The taxonomic status of this species is open to question. Mrs. Biggar proposed to place it in a new genus but it should probably be placed in an existing genus. It seems to be related to Lembus, but species of that genus have two oral membranes (Hoare, 1927), while only one has been made out for the present species. A single membrane is said to characterize Cyclidium, but the species under discussion lacks several of the characters of that genus, such as the existence of an extensive peristome. The genus Anophrys was established by Cohn (1866) for *A. sarcophaga,* which resembles the present species somewhat, but the form referred to by Rees (1884) as *A, sarcophaga,* shows a much closer resemblance. Di Mauro (1904) has described *Anophrys echini* from *Strongylocentrotus lividus* and *Sphaerechinus granularis,* but he states that it lacks a caudal filament. However, such a filament is easily overlooked, and his figure shows a membrane-like row of cilia along the right side of the peristome. Russo (1914) suggests that Di Mauro's species might belong in the genus Lembus, apparently overlooking the fact that Lembus has two oral membranes. The species found by Mrs. Biggar is larger, relatively thinner, and more definitely narrowed for the anterior third of the body, as compared with Di Mauro's *Anophrys echini,* hence her new species name, *elongata,* is employed.

Mrs. Biggar also found and partially described a ciliate which she identified as the one designated "A" by Jacobs. It is somewhat more than twice as long as broad, much flattened, rounded at both ends, with a posterior contractile vacuole and an oral cleft near the anterior end. The macronucleus is anterior to the middle and the region anterior to it is filled with refringent granules. Size: 35 by 16$\mu$. From the partial description and sketches furnished by Mrs. Biggar, one discerns a resemblance to the form called *Cryptochilum boreale* by Hentschel (1924), but it is much smaller. It is probable that neither the present species nor that of Hentschel belongs to the genus Cryptochilum, but since no name for it was given in Mrs. Biggar's notes, and since its characters are insufficiently determined, it will be left for future workers to study and give an appropriate taxonomic status.

In going over material obtained from the digestive tract of sea urchins from the coastal waters of North America and Bermuda, one is impressed with the considerable number of ciliates which are found as endozoic associates of these hosts, and also with the fact that many of them are so closely related to free-living species. The entire situation promises to provide very interesting data bearing on the question of the origin of the endozoic habit of such ciliates.]

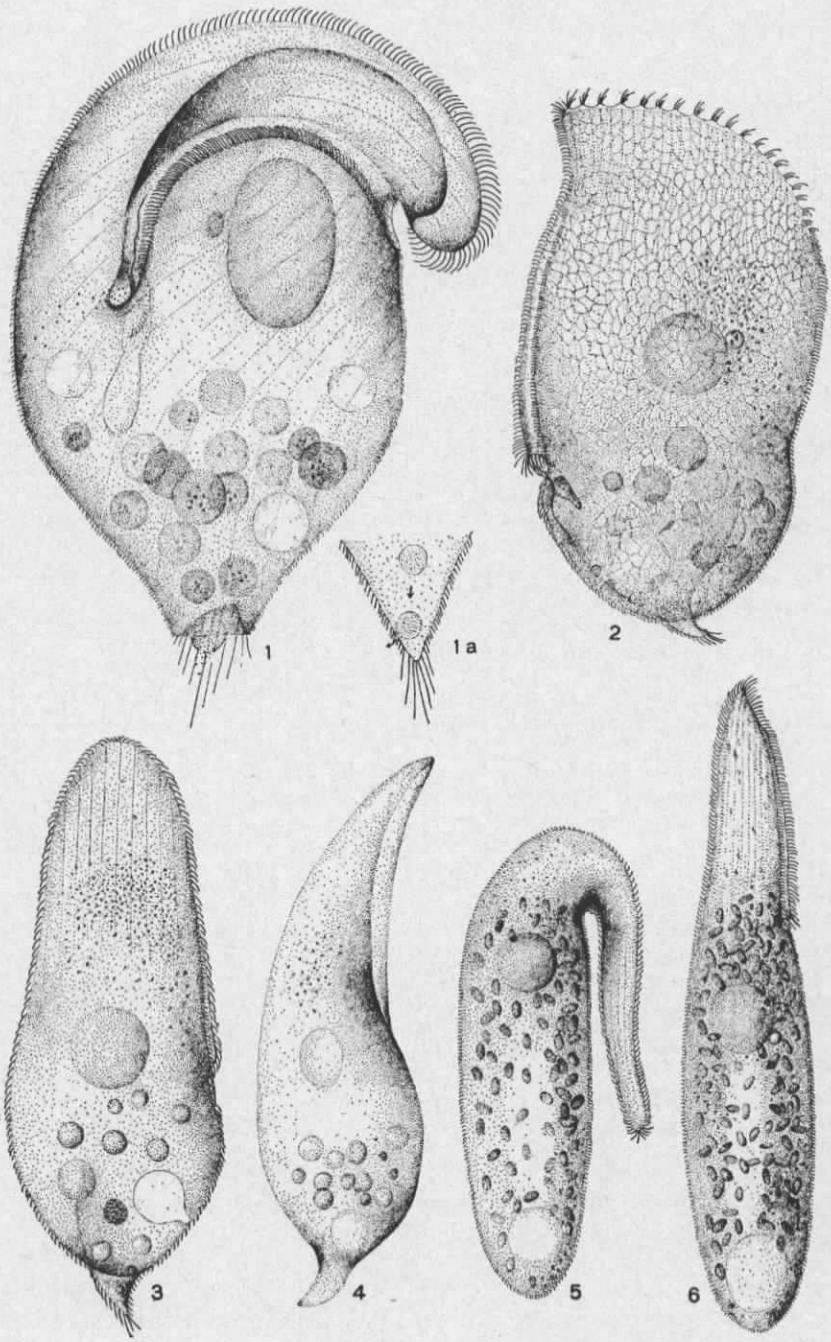


PLATE XXIII

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## Explanation of Plate XXIII

All figures, except 3 and 4, are from drawings made originally at a magnification of 1000 diameters; figures 3 and 4 are from drawings made at × 2000; all are reduced in printing to about one-half the original diameter. Figures 1 to 6 are from drawings made by Mrs. Biggar from fresh material. Figure 1a was copied from an original drawing by Mrs. Biggar.

Fig. 1.—*Metopus circumlabens*, ventral view; posterior end invaginated, as during egestion.

Fig. 1a.—*Metopus circumlabens*, ventral view; posterior end under normal conditions, as arrows show path of movement of food vacuoles to anal aperture.

Fig. 2.—*Cryptochilum bermudensis*, side view, showing general features of organization.

Fig. 3.—*Cryptochilum echinometris*, side view, showing general morphology.

Fig. 4.—*C. echinometris*, edge view, showing scoop-like profile.

Fig. 5.—*Anophrys elongata*, edge view, anterior end bent backward, showing flexibility of this region.

Fig. 6.—*A. elongata*, side view. Showing general morphology.

# ON THE ANATOMY AND SYSTEMATIC POSITION OF THE CAUSATIVE AGENT OF SO-CALLED SALMON POISONING

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Donham (1925) and later Donham, Simms and Müller (1926) described a fatal disease of dogs on the Pacific Coast of North America following the ingestion of uncooked salmon. These authors emphasized the constant association of the disease with a small intestinal trematode which they considered as the causative agent. The metacercariae of this parasite were found in the muscles and various organs of several species of salmonid fishes of the genera Salmo and Oncorhynchus caught in North American waters.

The trematode was identified the following year (1926) by Chapin who considered it to be a new species of the family Heterophyidae, and named it *Nanophyes salmincola*. The generic name proved however preoccupied and was emended by Chapin (1927) to Nanophyetus.*

Subsequently *Nanophyetus salmincola* was found by Cram (1926) in coyote (*Canis lestes*), raccoon (*Procyon psora pacifica*) and a lynx (*Lynx fasciatus fasciatus*), but it did not appear to be as pathogenic for these animals as for the dog.

The only complete description of this trematode accompanied by a diagram of the anatomical structure is that given by Chapin (1926). While studying the original material † for the revision of Heterophyidae, I found some discrepancies between its anatomy and the previous description and illustration. It appears that *Nanophyetus salmincola* has no genital sucker or similar organ and no seminal receptacle and has a conspicuous cirrus pouch. The eggs proved to be much smaller than the suckers and not the contrary, as is shown on Chapin's illustration.

Skrjabin and Podjapolskaja (1931) described *Nanophyetus schikhobalowi* from the aborigenes of East Siberia. This species was

* Some confusion exists with regard to the author's name for this genus. I have to thank Mr. Hall for permission to publish the following explanation: "under the impression that Chapin's name Nanophyetus had been published I referred to it in my paper which appeared early in 1927, a few months before Chapin's proposal of the name appeared. Consequently the name Nanophyetus should be cited as Nanophyetus Chapin in Hall, 1927."

† I have to thank Dr. A. Hassall of the Bureau of Animal Industry, Washington, D. C., for his courtesy in sending me several specimens of *Nanophyetus salmincola* obtained experimentally.

distinguished from *Nanophyetus salmincola* by the smaller size of the eggs as compared with the original description of Chapin and in some minor features, which can be attributed to age or the influence of fixation, but have no specific value.

A re-examination of the American material at my disposal showed that anatomical details in *Nanophyetus salmincola* are more variable than is mentioned by both Chapin and Skrjabin and Podjapolskaja, and that *Nanophyetus schikhobalowi* is identical with *N. salmincola.* The substantial difference between the description of Skrjabin and Podjapolskaja and our data consists in that in *N. schikhobalowi* no cirrus pouch and no esophagus were observed, while in *N. salmincola* both are conspicuous. In my opinion Skrjabin and Podjapolskaja's description is incomplete, for it is hardly possible, that two species similar in every other detail should differ so fundamentally in the structure of these organs.

The following is the description of *Nanophyetus salmincola,* obtained in America from experimental dogs *:

The worms are pyriform, slightly flattened dorsoventrally, 0.8 to 1.1 mm. long and 0.3 to 0.5 mm. wide (0.5 by 0.3 to 0.4 mm.). The oral sucker is 0.15 to 0.18 mm. in diameter (0.08 to 0.1 by 0.12 mm.) and opens subventrally. It is followed by a pharynx 0.06 mm. long (0.04 to 0.07 mm.) which is continued into an esophagus 0.06 to 0.07 mm. long. The ceca are much wider than the esophagus. They extend up to the level of the posterior part of the testes and lie between the testes and dorsal surface of the body. They present the shape of a horseshoe with a wide arc and narrower base. The ventral sucker is 0.12 to 0.13 mm. in diameter (0.11 to 0.12 mm.) and lies in the middle of the ventral surface of the body or a little in front.

Two large oval testes 0.2 to 0.3 mm. long (0.17 to 0.2 mm.) are situated symmetrically at the sides in the posterior half of the body. They lie obliquely to the long axis of the body. The cirrus pouch is situated behind the ventral sucker and to the left of the ovary. It is relatively large, up to 0.2 mm. long, has thin walls and contains a pars prostatica and a large seminal vesicle divided in two parts by a constriction.

The ovary is almost round, 0.07 to 0.11 mm. in diameter (0.04 to 0.08 mm.) and is situated on the right side in the intercecal space. A seminal receptacle is not in evidence. Laurer's canal opens dorsally on the right side at the level of the esophagus. The uterus consists of two coils directed posteriad which, when seen from the side, form a W. It opens into the tube-like genital sinus which is situated near

* The numerical data of Skrjabin and Podjapolskaja, which do not conform with ours are given in brackets.

the posterior edge of the ventral sucker. The whole course of the uterus lies in the sagittal plane of the body and therefore in mounted specimens it appears as a short row of eggs, 10 to 15 in number, lying between the testes. The eggs are oval, 64 to 80$\mu$ long and 34 to 50$\mu$ wide measured in mounted specimens. The vitellaria consist of irregular follicles scattered under the dorsal surface of the body, except in the anterior region which is free. They are often very thick and obscure other anatomical details in mounted specimens. The excretory vesicle is not well seen in our material but seems to be sac-shaped and is situated behind and between the testes.

There exist some discrepancies with regard to the systematic position of *Nanophyetus salmincola*. As already mentioned Chapin ascribed it to the family Heterophyidae. In an earlier paper (1929) the present author emphasized, on the basis of re-examination of the original material, that Nanophyetus does not belong to this family. However, Skrjabin and Podjapolskaja (1931) accepted Chapin's opinion. The most characteristic features of the Heterophyidae are: absence of a true cirrus pouch, presence of a large seminal receptacle and of peculiar, for the most part armed, genital papillae or gonotyls. All these features are absent from *Nanophyetus salmincola*. The author is of the opinion that the genus Nanophyetus corresponds exactly to the genus Troglotrema Odhner 1914, of the family Troglotrematidae Braun 1915 and should be regarded as a synonym. *Nanophyetus salmincola* should therefore be renamed *Troglotrema salmincola* (Chapin, 1926). Another species of this genus, *T. acutum* (Leuckart), has been recently redescribed and illustrated by Baer (1931). *Macroorchis spinulosus* Goto in Aado, 1919 (see Dollfus, 1925:197), is probably a third species, but this can only be determined through a restudy of this species which is insufficiently described. The two certain species may be distinguished by the following differences:

| | *T. acutum* | *T. salmincola* |
|---|---|---|
| Ceca..... | Sinuous and extend beyond the testes | Smooth and do not reach the posterior extremities of the testes |
| Uterus..... | Makes longitudinal and transverse loops | Loops only in the sagittal plane |
| Localization..... | Frontal sinuses of polecats..... | Intestine of carnivora and man |

The description of the genus Troglotrema was given by Odhner (1914) and was based on contracted specimens of *T. acutum*. If the above-mentioned description of *T. salmincola* and the description of *T. acutum* given by Baer (1931) be taken into consideration, the generic diagnosis of *Troglotrema* can be emended as follows:

## TROGLOTREMATIDAE

Both suckers well developed. Testes and ovary with smooth outlines. Cirrus pouch well developed, containing double seminal vesicle and pars prostatica. Seminal receptacle very small or absent. Uterus confined within the intercecal space. Vitellaria very well developed, under the dorsal surface of the body. Both male and female organs open in a deep genital sinus devoid of any additional structures and located behind the ventral sucker. Free in the intestine or frontal cavities of mammals.

Type species: *T. acutum* Leuckart, 1842.

Besides the genus *Troglotrema* three more genera belong to the family Troglotrematidae: Paragonimus Braun, 1899, Renicola Cohn, 1904, and Collyriclum Kossack, 1911.* They may be distinguished according to the following key:

A. Living in crypts or cysts; ovary lobate;
   1 testes lobate:
      a, uterus occupies almost entire body, forms uterine sac ..... RENICOLA †
      b, uterus occupies a small space in center of body, does not form uterine sac ..... PARAGONIMUS
   2 testes with smooth outlines ..... COLLYRICLUM
B. Living free in open cavities or intestine of mammals; neither ovary nor testes lobate ..... TROGLOTREMA

The inclusion of the causative agent of the so-called salmon poisoning in the genus Troglotrema is important not only from the zoological standpoint but for human hygiene, for this parasite has been found in man. Its inclusion in the Heterophyidae, as suggested by previous authors, would indicate that the parasite is comparatively little harmful. In view of its inclusion in the genus Troglotrema its pathological significance, though still little known, may a priori be considered as serious. As a matter of fact, for dogs *Troglotrema salmincola* is a very dangerous parasite. Hoeppli (1926) described severe histological changes of the intestinal mucosa in dogs caused by this parasite. Donham, Simms and Müller (1926) give, inter alia, the following details on the disease produced experimentally in dogs by feeding them on salmon infected with metacercariae of *Troglotrema salmincola*:

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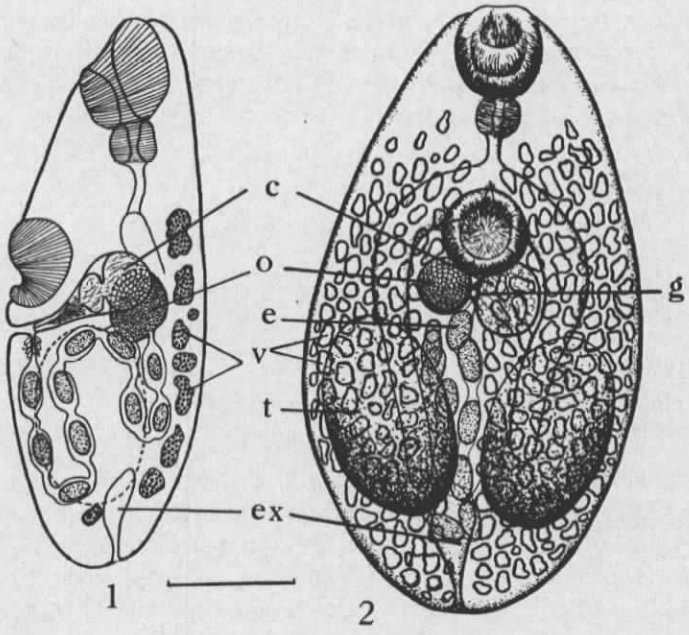
* Odhner (1914) and Jegen (1917) also ascribe the genera Pholeter Odhner, 1914, and Brandesia Stossich, 1899, to Troglotrematidae. However, I regard the genus Pholeter as synonym of the genus Collyriclum and I agree with Poche (1926) in excluding the genus Brandesia Stossich from Troglotrematidae.

† Most of the species of the genus Renicola are insufficiently described. The generic characters of Renicola used in this key are based on the description of *R. glandoloba* Witenberg, 1929a.

"The symptoms do not develop until 7 to 10 days after the dog has eaten the fish . . . the onset is very sudden, the temperature rises to 105 to 107 F. . . . symptoms usually last for 24 to 48 hours after which the temperature is gradually lowered. At this time the diarrhea develops which is blood-tinged at first and later is practically all blood. . . . In about 6 to 8 days the temperature usually becomes subnormal and death occurs 24 to 48 hours later. An occasional case recovers . . . 1 gram of infected salmon may sometimes produce death. . . ."




EXPLANATION FOR TEXT FIGURE

*Troglotrema salmincola.* 1. Sagittal section (semidiagrammatic); 2. ventral view. Scale equals 0.2 mm. *c*, cirrus pouch; *e*, egg; *ex*, excretory bladder; *g*, genital pore; *o*, ovary; *t*, testis; *v*, vitellaria.

One may suppose that in man the parasite produces similarly serious symptoms, but so far nothing is known about them.

It is noteworthy that the second species of this same genus, *T. acutum*, produces serious pathological changes in the frontal sini of polecats. According to Olt (1929) the parasites are "in direct contact with the blood vessels and produce decalcification of the wall of the sinus. The wall becomes membraneous at the site where the parasites fix themselves and this results in the formation of openings with irregular edges, visible on the prepared skulls" (Translated from Baer, 1931).

It may be mentioned here, that some other species of the same family are known as injurious parasites of man (*Paragonimus ringeri*) and poultry (*Collyriclum faba*).

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# LIFE HISTORY OF THE NORTH AMERICAN LUNG FLUKE OF MAMMALS*

DONALD J. AMEEL

On July 29, 1931, a collection of snails of the species *Pomatiopsis lapidaria* taken near Ann Arbor, Michigan, yielded several individuals shedding microcercous cercariae closely resembling the cercaria of the human lung fluke, Paragonimus. Numerous cercariae were placed in a dish with parasite-free crayfishes. They soon disappeared from sight and an examination of the ventral side of a crayfish revealed numerous cercariae penetrating through the thin chitin of the tail at the union of the segments. About twenty hours after exposure, eleven cercariae were found in the heart muscles of one of these crayfishes. Later examination of other crayfishes of the same lot revealed similar infections. The cercariae did not encyst immediately, but individuals were found one to two days after infection completely enclosed in a very thin membrane. After a four weeks sojourn in the crayfishes, encysted metacercariae were recovered that agreed in every respect with proven Paragonimus metacercariae found in naturally infected crayfishes of the same region.

Although this cercaria differs in certain details from Kobayashi's figures and descriptions of the Asiatic lung fluke, there are many points of similarity. A comparison of the work of Kobayashi (1921) and of Faust (1929) on the same species shows several discrepancies. My material agrees much more closely with Kobayashi's figures than with Faust's. Measurements in microns were made of fifteen specimens killed in 5% formalin and slightly flattened under a number one cover slip. The averages are as follows: total length, 178; width, 93; tail, 15 by 14 (often perfectly spherical); oral sucker, 48 (usually slightly elongated anteriorly); stylet, 39 by 7; acetabulum, 47 by 34. The cercaria is completely covered with spines of about equal length which are most conspicuous on the posterior portions of the body and tail. The group of large spines located on the tip of the tail is particularly conspicuous, for the remainder of the tail is sparsely covered with minute spines. Penetration glands surround the lateral and anterior edges of the acetabulum but usually do not extend beyond its posterior border. These can be differentiated into two types by their affinities for neutral red: a lateral group of four large cells on each side of the body which have a great affinity for this stain, and a median mass of six smaller cells which stain poorly. Four large ducts leave each group of

* Contribution from the Department of Zoology, University of Michigan.

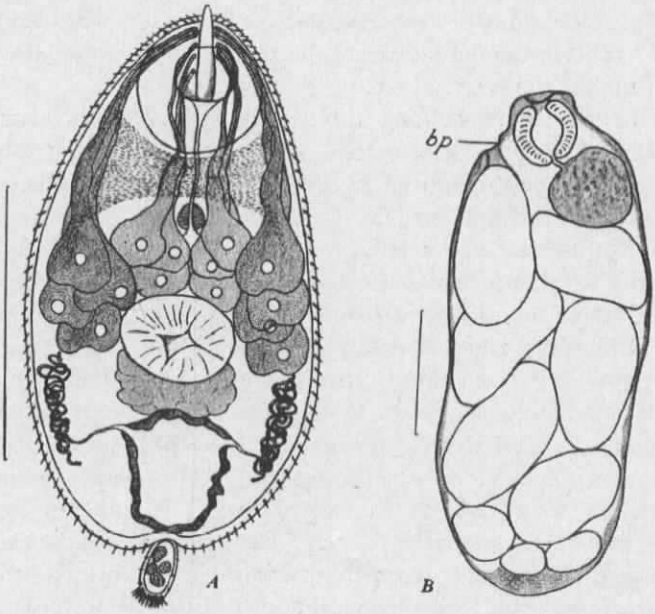
lateral cells, proceed along the edge of the cercaria, penetrate the oral sucker, and open on each side of the stylet. Six ducts of about the same size as the lateral ones arise from the central cells, proceed forward in the mid-region of the cercaria in two groups of three each and penetrate the oral sucker, opening three on each side of the stylet just posterior to the openings of the lateral ducts. A long prepharynx is present, and a pharynx is located about midway between the oral sucker and the acetabulum. The remainder of the digestive tract is still undifferentiated. The brain is a conspicuous butterfly-shaped mass situated between the oral sucker and the pharynx, extending to the ducts of the lateral penetration glands. The genital primordium is represented by a mass of cells posterior to the acetabulum. A median, thick-walled excretory bladder about as broad as the acetabulum occupies the region between the latter and the posterior end of the body.

One of the most striking differences between this cercaria and Kobayashi's is the character of the penetration glands and their ducts. The only ducts described and figured by him lie in the lateral fields and arise from groups of fairly small cells of unknown number. Another feature of Kobayashi's cercaria is a group of cells larger than the penetration glands, which Kobayashi calls "gland-like cells." These cells are located along the median ventral part of the body and fill up the regions anterior and posterior to the acetabulum. Kobayashi did not note ducts arising from these cells. Judging from Faust's figure and this material, these may prove to be penetration glands. The genital primordium of Kobayashi's cercaria is less than twice that of the acetabulum while this cercaria has an oral sucker with a diameter not much greater than that of the acetabulum. In other respects, the cercariae resemble one another closely. However, no precise comparison can be made, for Kobayashi apparently worked with preserved material, while the present study was made almost exclusively on living material with the aid of *intra vitam* stains.

The cercaria described by Faust presents far greater differences from my material than does that described by Kobayashi. Faust pictured two types of penetration glands: two median groups of five large cells each, extending almost to the posterior end of the body; and two distinctly different groups, each consisting of four considerably smaller cells located between the larger cells and the margin of the body. All the ducts from these cells are located close to the median region. However, the most noteworthy differences are the location of the pharynx, which is shown immediately adjacent to the oral sucker, and the presence of pharyngeal glands with ducts opening into the pharyngeal region. The genital primordium is even more extensive than that shown in Kobayashi's figures. Faust says that the cercariae "swim around in the water." These cercariae are notable in their

inability to swim. They generally creep along a surface like a leech or, if the water is agitated sufficiently to cause them to lose their hold, they float, executing the same type of movement.

The rediae are located in the liver of the snail host. Their shape is that of an elongated ellipsoid, devoid of appendages. The cuticula is thrown into minute rugae over most of the body. The mouth is terminal, opening immediately into a large, spherical, muscular pharynx. A short esophagus leads into a short, usually spehrical gut which is often smaller than the pharynx. The gut wall consists of a single layer of large, clear cells. Amber colored particles derived from the




Textfigure A.—Camera lucida drawing of a cercaria. Scale, 0.10 mm.

Textfigure B.—Camera lucida drawing of a redia, *b p*, birth pore. Scale, 0.10 mm.

tissues of the snail fill the lumen of the gut. The entire digestive tract is generally confined to the anterior third or fourth of the body. The remainder of the body cavity of the redia is filled with cercariae in various stages of development. A birth pore is located in the margin of the body adjacent to the pharynx. Germ balls lie in the posterior portion of the body. Average measurements of nineteen rediae slightly flattened under a number one cover slip are: length, 564; width, 226; pharynx, 76; gut, 70$\mu$. The redia described by Kobayashi has a long, fairly narrow gut extending posteriorly to the middle of the body. Kobayashi does not mention a birth pore.

The differences represented by the figures of the three cercariae under discussion, if valid, together with differences in the rediae, are sufficient to distinguish at least two species of lung fluke, an Asiatic and a North American, and the differences in the descriptions given by Kobayashi and Faust may indicate two species of the Asiatic lung fluke. However, a more thorough study of these cercariae is necessary before this species question can be definitely settled.

Metacercariae found in local crayfishes resemble those described and figured by Kobayashi and Faust. However, their location in the tissues of the Asiatic and North American hosts is decidedly different. Metacercariae in all the crayfishes I have examined were usually present in the heart tissue and occasionally in adjacent tissues but never in the gills or body muscles which are the parts commonly infected in the Asiatic crabs and crayfishes. This may prove to be another character for the differentiation of species. I have found metacercariae in *Cambarus propinquus, C. robustus, C. virilis, C. diogenes,* and *C. rusticus,* and have raised them experimentally in *C. immunis.* There is little doubt that they will develop in any species of crayfish exposed to infection.

Though dogs, cats and swine have been found infected with this worm, the mink has been proved by Wallace (1931) to be the true definitive host. Racoons eat large quantities of crayfishes but I have definitely proved by experiment as well as by examination of 308 carcasses that they are immune to infection.

Normally the definitive host becomes infected through the eating of crayfishes bearing the metacercariae, but it is also possible for the transmission of very small worms from one definitive host to another. Yokogawa and Suyemori (1920) experimented with this type of transmission but for some reason had no success. However, they cite the work of Kawamura and Ando, both of whom report positive results. Neither of these papers is available.

White rats and domestic cats were used in the following experiments. For some reason, as yet unexplained, worms from one to one and a half millimeters long remain in the pleural and abdominal cavities of white rats for months without penetrating the lungs. Such worms even after one and one-half months still retain their stylets. No stylets were found in worms older than these. Twelve 185-day old worms from the pleural cavities of several white rats were fed to a cat known to be previously uninfected. Seven weeks later, six adult worms were recovered from the lungs. Experiments involving worms after shorter periods of time in pleural and abdominal cavities of white rats were equally successful. However, negative results were secured by feeding twenty-four worms (two to three millimeters long), obtained from the

lungs. It has not been possible to determine that these worms linger in the body cavities of minks as they do in rats; nevertheless, the above experiments are proof enough that worms which have not entered the lungs are infective if eaten by another host. Thus, a mink during the first few weeks of infection is probably just as effective a carrier as a crayfish. I have collected information from trappers who have seen dogs and cats eat minks, and I have also induced cats to eat them. Two sufficiently starved laboratory cats willingly ate crayfishes, definitely proving that they may also become infected directly by the eating of infected crayfishes. I have no information on the eating of crayfishes by dogs.

I wish here to express my grateful appreciation to Professor George R. La Rue, under whom this investigation is being undertaken. I am also indebted to Dr. Helen F. Price for aid in the collection of host material.

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# THE APPEARANCE AND SIGNIFICANCE OF THE UNFERTILIZED EGGS OF *ASCARIS LUMBRICOIDES* (LINN) *

G. F. Otto

Muira and Nishiuchi as early as 1902 gave a rather comprehensive discussion of the appearance and occurrence of the unfertilized egg of *Ascaris lumbricoides*. Despite the fact that these authors as well as most subsequent texts in parasitology have figured the unfertilized eggs they are not generally recognized by laboratory technicians making fecal diagnoses. In fact many parasitologists seem to consider them only of passing interest. The above authors also described the asymmetrical, often triangular type of egg, but it has never, to my knowledge, been figured and consequently is rarely recognized by anyone doing routine fecal examinations.

It is scarcely necessary to point out that when unfertilized eggs alone appear in the feces their recognition is important. With the development of quantitative methods of fecal diagnosis, whereby an attempt is made to estimate the number of worms present by counting the eggs found in a measured portion of the stool, the recognition of these eggs when they appear mixed with the fertilized eggs also becomes important.

Though the commoner unfertilized eggs have been described and often figured it seems worthwhile to review and describe them here. The figures and descriptions presented in this paper are principally from eggs observed in the N/10 (0.04 per cent) sodium hydroxide of the Stoll dilution egg counting preparation. Some eggs, however, were studied in simple saline-feces smears and found to be essentially the same as those in the N/10 sodium hydroxide. Figures 3, 4, 5 and 6 represent the commoner forms of unfertilized ascaris eggs. The albuminous outer coat (alb.) may or may not be present on eggs taken from the uterus (Wharton, 1915), found in the simple feces smear or seen in N/10 sodium hydroxide. The sodium hydroxide acts as a slow solvent of the albuminous coat distending it during that process. It is, therefore, not uncommon to find the unfertilized ascaris eggs in this medium having what appears to be an irregular wide, either transparent or bile stained, halo around the shell. This is well illustrated in figures 5 and 8 while figure 3 shows the albuminous coat distended slightly beyond normal. This is much less frequent in the normal fertilized eggs.

* From the Department of Helminthology of the School of Hygiene and Public Health of the Johns Hopkins University.

The writer is indebted to Dr. W. W. Cort for helpful criticisms and to Dr. John Stumberg for most of the photomicrographs.

No nucleus is apparent in the unfertilized eggs and the contents of the shell are never organized but rather are disintegrated and appear as finely granular or as larger globules of fat or yoke. These contents may be uniformly distributed or collected at one end or one side of the egg.

Muira and Nishiuchi (1902) dealing with these unfertilized eggs and Foster (1914) considering the unusually large fertilized eggs sometimes seen have pointed out that the diameters of all the different types of eggs of *A. lumbricoides* are essentially the same, the variation in size being primarily in length. The first authors report the average diameter of two groups of unfertilized eggs as 0.040 and 0.050 mm., the maximum and minimum diameters in the first group being 0.037 and 0.044 mm. The average lengths for these same groups were 0.076 and 0.0809 mm., the limits in the first group being 0.067 and 0.086. Foster considering the abnormally large fertilized eggs also found that the diameter averaged about 0.050 mm. The writer measured 30 unfertilized eggs from 20 different stools and found the maximum, average, and minimum diameters to be 0.041, 0.048, and 0.049 mm. and the lengths 0.066, 0.077 and 0.106 mm. without the outer albuminous layer of the shell. The widths are well within the range of normal fertilized eggs.

While the eggs shown in figures 3, 4, 5 and 6 are by far the most common type of unfertilized ascaris eggs seen there are a number of eggs which are so atypically shaped as to be passed unnoticed except by the most practiced observer. Figures 7, 8, 9 and 10 are representative of some of these types. It is not unusual to find these eggs alone in stools having a low egg count. Only one or two, or occasionally a larger percentage, may be also found per slide * in heavy infestations. Except for shape their appearance is similar to that of the more typical unfertilized eggs. The shortest axis of these eggs is often greater than the diameter of the fertilized or of the more common unfertilized eggs. The longest axis, however, is rarely as great as the length of the more common unfertilized eggs. These eggs are more often than not triangular shaped as viewed under the microscope (figures 7, 8 and 9). That they are not always triangular is readily seen when they are rolled over. A bulge then appears on one surface but the egg can be placed so that it appears to be symmetrically formed around one major axis. About one to five per cent of the stools containing ascaris eggs were seen to have these triangular shaped eggs or eggs with bulges. In a number of cases these alone appeared on slides showing five or less eggs. Some of the eggs which appear symmetrical when seen in routine examination may also appear oddly shaped when viewed from a different

* Stoll dilution egg counting method wherein the eggs are counted in the small drop (0.075 cc.) of a 1 to 15 dilution of feces in N/10 sodium hydroxide (Stoll, 1923; Stoll and Hausheer, 1926).

angle. The finding of these eggs in N/10 sodium hydroxide solution suggested the possibility that this medium might be in some way exerting a distorting influence. However, Muira and Nishiuchi reported them from feces before the use of the Stoll method and from the uteri of adult worms and I have since found them under similar conditions.

To determine the relative importance of the unfertilized eggs diagnostically differential counts were made on ascaris eggs found in the Stoll small drop preparation from 820 stools. All types of unfertilized eggs were grouped together under one heading and considered in relation to the normal fertilized eggs. A total of 51,329 ascaris eggs were seen in these 820 preparations, an average of 63 eggs per slide of which 8155 or 15.9 per cent were unfertilized. Of these unfertilized eggs 2585 were found on 214 slides on which no fertilized eggs were

TABLE 1.—*Relative Distribution of Fertilized and Unfertilized Eggs in Stoll Small Drop Preparation of 820 Stools. The Preparations Are Grouped According to the Percentage of Fertilized Eggs Present and the Number and Percentage of Slides in Each Group Recorded*

| Per Cent of Eggs Fertilized | Number of Slides in Each Group | Percentage of Slides in Each Group |
|---|---|---|
| 0 | 214 | 26 |
| 1–10 | 16 | 2 |
| 11–20 | 14 | 1.5 |
| 21–30 | 14 | 1.5 |
| 31–40 | 9 | 1 |
| 41–50 | 22 | 3 |
| 51–60 | 25 | 3 |
| 61–70 | 17 | 2 |
| 71–80 | 37 | 5 |
| 81–90 | 66 | 8 |
| 91–99 | 126 | 15 |
| 100 | 260 | 32 |
| Total | 820 | 100 |

to be seen (Table 1), an average of 12 eggs per slide. However, most of the slides, whereon unfertilized eggs alone gave evidence of infestation, had less than 12 eggs per slide; 72 per cent having 10 or less and 51 per cent five or less. However, a number did have rather heavy infestations. There were nine cases in which 50 or more unfertilized eggs were the only evidence of heavy infestation. These counts were 53, 59, 62, 73, 76, 77, 128, 151, 202. There were still a larger number of high egg counts in which most of the eggs found were unfertilized. There were 13 cases having from 50 to over 200 eggs per slide in which over 50 per cent of the eggs were unfertilized. Table 1 gives the distribution of cases according to the relative number of fertilized and unfertilized eggs present. The percentage having only unfertilized eggs, the two types mixed and only fertilized eggs (26, 42 and 32 per cent) is in general like that reported by Muira and Nishiuchi in 1902. They found that of the 35 cases seen 14 or 40 per cent had only unfertilized eggs; 4 or 14.3 per cent were mixed and 15 or 45.7 per cent had only fertilized eggs. From Table 1 it will be seen that

in only 48 per cent of the cases were 90 per cent or more of eggs fertilized while in 35 per cent of the cases all or more than half of the eggs were unfertilized. In the nine per cent of the cases where both types were present but more than half of the eggs were unfertilized the average egg count was 41, compared with the average count of 63 eggs per slide for the whole series of 820 cases.

The significance of the unfertilized eggs diagnostically has been demonstrated and it is interesting to consider their significance biologically. That these eggs are unfertilized eggs of *A. lumbricoides* and not degenerate eggs or eggs of another species is well established. Muira and Nishiuchi and others including the writer have removed single females or small numbers of females and no males from individuals whose stools contained only these eggs. The uteri of such females always contained these characteristic eggs. Muira and Nishiuchi and subsequent workers have studied the reproductive tract of females which had been associated with males. The lower part of the uteri usually contain either only the normal fertilized eggs or both fertilized and unfertilized eggs. In the vicinity of the seminal receptacle those eggs which later develop as the normal fertilized eggs are found surrounded and being penetrated by spermatozoa. Other eggs in which no spermatozoa can be demonstrated retain the characteristics of these unfertilized eggs as they pass down the uteri.

As already mentioned, solitary females, or several females without males, are found when a person passing only unfertilized eggs is treated. This may be due to the females never having been in copula or to the supply of spermatozoa received by copula having already been exhausted. It is difficult to determine the exact conditions of development but it seems probable in many cases that males have never been present with these isolated females. Perhaps in other cases males did develop but were dislodged without ever having functioned. Proof that copulation takes place more than once during the life of the worm is equally difficult to obtain. However, Looss (1905: 113) and Herrick (1928: 139-140) both suggest from experimental evidence with hookworm that it does take place more than once. Thus a female probably does not receive and store in the seminal receptacle enough spermatozoa from one copulation for the fertilization of all the eggs to be produced. Hence if a solitary male were to be dislodged after its first copulation the female might easily use up its supply of spermatozoa after a time and produce only unfertilized eggs thereafter. It is interesting to consider what has happened in those few cases mentioned where many unfertilized eggs alone, up to several hundred were found per slide, indicating about 20 or more females. No such cases were treated and hence one can only conjecture a shortage of males due probably both to shortage in the original infection and an early dislodgement of those which developed.

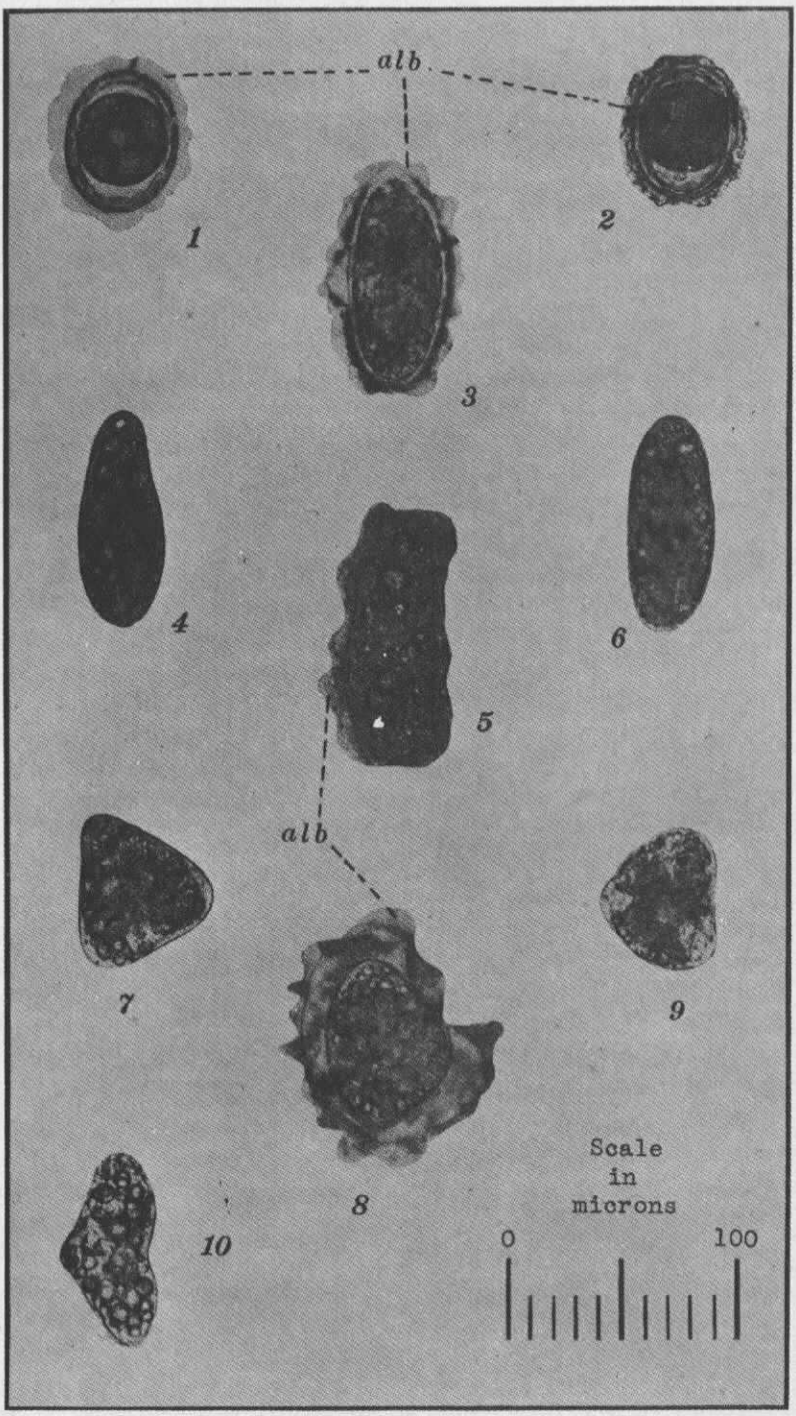


PLATE XXIV

When both fertilized and unfertilized eggs are found in the same infestation it is probably due in some cases to the presence of some females laying just unfertilized eggs and in other cases to the laying of both types of eggs by the same worm. The writer has found both types of eggs in the uteri on dissection and Muira and Nishiuchi used such worms for the basis of the histological studies on the nature of the unfertilized eggs. The proportion of worms laying both types of eggs and laying only one type must vary in the different individuals from day to day and any attempt to arrive at such a proportion from the egg count would be little more than a guess.

## SUMMARY

Unfertilized eggs were found to represent 15.9 per cent of 51,329 ascaris eggs seen in 820 small drop preparations of the Stoll dilution egg count method. These eggs were found alone in 26 per cent of the slides, mixed in 42 per cent and absent in 32 per cent. They were distributed in high and low egg counts but most of the slides having just unfertilized eggs had low egg counts, the average being 12 and 72 per cent having less than 10 eggs, whereas the average egg count for the entire 820 slides was 63. Special attention is called to the asymmetrical unfertilized eggs and all are figured. Single female worms may be producing either only unfertilized or fertilized eggs at one time or both at the same time. Inasmuch as fertilization probably takes place more than once during a life time the production of worms producing one or both types of eggs must vary from time to time.

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## EXPLANATION OF PLATE XXIV

Photomicrographs of eggs of *Ascaris lumbricoides* (Linn.) × 330.

Abbreviation: alb., albuminous outer layer of shell.

Figs. 1, 2.—Normal fertilized eggs.

Figs. 3, 4, 5, 6.—Common types of unfertilized eggs.

Figs. 7, 8, 9, 10.—Less common types of unfertilized eggs.

# OBSERVATIONS AND EXPERIMENTS ON THE OPALINID CILIATES OF THE GREEN FROG*

ROBERT HEGNER

This is a continuation of studies begun a number of years ago (Hegner, 1922) because of the fact that the tadpole of green frogs and bull frogs have a high incidence of infection with opalinids, whereas the adults are usually free from these ciliates, although both tadpoles and adults of other American frogs are infected. The chief questions involved are (1) when and why do green frog tadpoles lose their opalinids and (2) why do not adult green frogs become reinfected? Green frogs, leopard frogs and tree frogs from Mount Desert Island, Maine, were studied during the summer of 1930 in an attempt to answer these questions.

Do adult green frogs on Mount Desert Island, Maine, harbor opalinids? This was the first problem undertaken. Ten adult green frogs of various sizes, and presumably of various ages, were examined. No opalinids were found. Ciliates of the genus Nyctotherus were present in the rectum of one specimen; trichomonads were present in large numbers in all; hexamitas were found in nine, but were few in number.

At what stage does the green frog lose its opalinids? To answer this question tadpoles were separated into three groups as follows: (1) without external legs, (2) with two posterior legs, and (3) with four legs. The lengths of body, tail and intestine were obtained for those with two and four legs and presented in the following table.

| Number of Tadpoles | Number of Legs | Length of Body | | Length of Tail | | Length of Intestine | |
|---|---|---|---|---|---|---|---|
| | | Range, Mm. | Average, Mm. | Range, Mm. | Average, Mm. | Range, Mm. | Average, Mm. |
| 15 | 2 | 35–40 | 36 | 50–105 | 84 | 33–55 | 43 |
| 20 | 4 | 35–45 | 38 | 3– 85 | 33 | 28–80 | 64 |

Every one of at least one hundred tadpoles without legs that were examined was infected with opalinids. All of the fifteen tadpoles with two posterior legs were likewise infected with opalinids. Large numbers were present in eleven of them and a few in the other four. Nyctotherus was noted in all but two and trichomonads and hexamitas in all. No opalinids nor Nyctotherus were found in the twenty tadpoles

* From the Mount Desert Island Biological Station at Salisbury Cove, Maine, and the Department of Protozoology of the Johns Hopkins School of Hygiene and Public Health.

with four legs. Eight of this group contained trichomonads and thirteen were infected with hexamitas.

These observations lead to the conclusion that green frog tadpoles loose their opalinids during metamorphosis between the two-legged and four-legged stages.

Do the tadpoles of other species of frogs retain their opalinids during metamorphosis or do they lose them at this time and become reinfected as young frogs? Data obtained in 1922 were inconclusive on this point. Three metamorphosing tadpoles of *Rana pipiens* and two of three young frogs of this species were found infected at that time. Five tadpoles of *Bufo americanus* with two visible legs were infected and five non-infected. In 1930, twenty-two tadpoles of *Rana pipiens* were examined for opalinids. Five tadpoles without legs were all well infected; eight with two legs were also well infected; and of nine with four legs, opalinids were found in three and absent from six. These results are also inconclusive. They indicate that the late stages in metamorphosis are unfavorable for the protozoan inhabitants of the rectum since Nyctotherus, trichomonads and hexamitas were also absent from three of this group. Probably opalinids are retained by some and lost by others, the latter becoming reinfected as adults.

The situation with respect to *Hyla versicolor* is more definite. Sixteen tadpoles of this species were examined, three with two small legs, three with two large legs, and ten with four legs. Every specimen contained a good infection with opalinids indicating that these ciliates remain present throughout the period of metamorphosis of this species.

Can recently metamorphosed green frogs be reinfected with opalinids from green frog tadpoles? Forty-seven frogs were used in an attempt to answer this question. Material containing opalinids taken from the rectum of green frog tadpoles was injected at once into the stomach of eight of these frogs. Opalinids and the other intestinal protozoa present in the inoculum were found in the stomach of one frog one and one-half hours after injection in apparently a normal condition. None had reached the rectum. Five frogs were killed and examined four hours after the injection. Opalinids were still present and viable in the stomach of two of these but none were found in the rectum. Two frogs were kept twenty-two hours before examination; these were both negative throughout for opalinids. It seems evident that trophozoites of opalinids from green frog tadpoles are unable to pass through the stomach and intestine and set up an infection in the rectum of young green frogs. This might have been possible if cysts had been used instead of trophozoites.

In thirty-nine of the young green frogs opalinids from green frog tadpoles were inoculated into the rectum and the frogs killed at intervals

of from 2 to 96 hours. Two of the frogs died; the data for the other thirty-seven are as follows:

| Number of Frogs | Interval Between Inoculation and Examination, Hours | Opalinids |
|---|---|---|
| 1 | 2 | Negative |
| 2 | 2.5 | Two positive; both with a few |
| 4 | 5 | Two positive; one with one, other with many |
| 4 | 21 | Two positive; one with two, other with few |
| 4 | 24 | One positive, with several |
| 8 | 48 | Two positive; one with two, other with several; all alive but not swimming about |
| 5 | 50 | One positive, with few quiescent |
| 4 | 72 | One positive; with two quiescent |
| 5 | 96 | Negative |

Although these opalinids were inoculated in large numbers directly into the rectum, which is their optimum habitat in other species of frogs, only eleven of thirty-seven retained them and they were few in number compared with their abundance in the inoculum. In four frogs the opalinids persisted in very small numbers for from forty-eight to seventy-two hours, but were not swimming about, although they were alive, as indicated by the movement of their cilia. Some of them were evidently abnormal. Under these conditions it seems impossible to colonize the rectum of recently metamorphosed green frogs with opalinids living normally in green frog tadpoles. It might be possible to induce other species of opalinids to live in the rectum of green frogs, but no attempt was made to do so.

Why do green frog tadpoles lose their opalinids at the time of metamorphosis and metamorphosed green frogs resist infection? In a previous report (Hegner, 1922) experiments on green frog tadpoles with different diets led to inconclusive results, although a largely meat diet and a diet of thyroid substance seemed detrimental to the ciliates. Whether the decrease in the number of opalinids under these conditions was due to the direct action of the diet or to the speeding up of metamorphosis could not be determined. It is suggested that diet is not the controlling factor but that digestive secretions produced by the green frog render the rectum an unfavorable habitat for the opalinids.

## CONCLUSIONS

Tadpoles of the green frog on Mount Desert Island, Maine, are heavily infected with opalinids, whereas newly metamorphosed frogs and older frogs are not. The tadpoles lose their opalinids during metamorphosis between the two-legged and four-legged stages. Some tadpoles of *Rana pipiens* seem to retain their opalinids during metamorphosis; others apparently lose theirs and become reinfected after metamor-

phosis. Tadpoles of *Hyla versicolor* appear to retain their opalinids throughout metamorphosis. The trophozoites of opalinids from the rectum of green frog tadpoles are unable to pass through the stomach and intestine of metamorphosed green frogs and reach the rectum in a viable condition. Cysts might be able to do so. Opalinids from green frog tadpoles injected directly into the rectum of metamorphosed green frogs are unable to set up infections. It is suggested that the intestinal secretions of the green frog render the rectum of this species unfavorable for opalinids.

## Literature Cited

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# ON THE PRESENCE OF PERIPHERAL CHROMATIN IN *ENDOLIMAX NANA*

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*Limax*-amoebae have been described from a large series of hosts, for example: oysters; cockroaches; termites; larvae of *Phyllophaga* sp., crane-flies and Harlequin flies; rock-fish; frogs; lizards; domestic fowl; domestic turkey; rats; man and others. In the descriptions of these various amoebae is found more or less general agreement regarding certain features of the nuclear structure. The karyosome is uniformly recorded as being of large size and staining intensely with hematoxylin. In some of the species spoke-radii are reported to connect the karyosome with the nuclear membrane. The greatest discrepancies, however, occur in the observations on the peripheral chromatin, some forms being recorded as having an abundance, while none was noted in others.

In the form from man, *Endolimax nana,* the nucleus is described as having a large, darkly staining karyosome, with spoke-radii extending to the nuclear membrane, on which is to be found no true peripheral chromatin. In view of the differences of opinion regarding the nuclear structure of the *limax*-amoebae, some observations on the nucleus of *Endolimax nana* seemed worthy of presentation. The writer is grateful to Dr. D. H. Wenrich of the Department of Zoology of the University of Pennsylvania, for guidance and criticism.

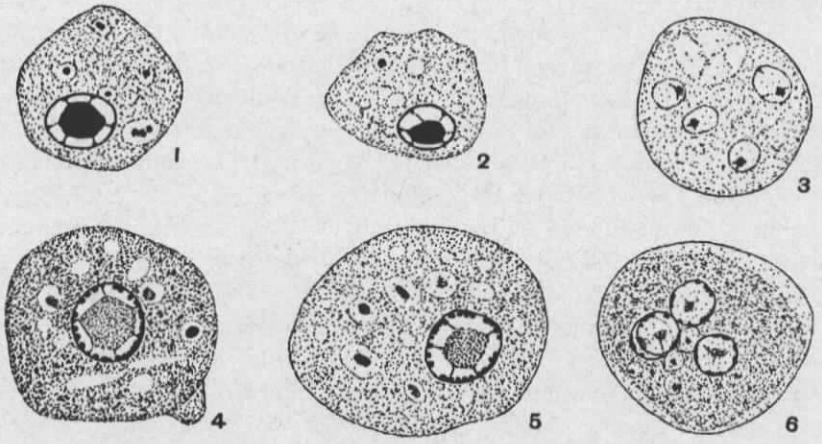
The material for these observations was obtained from numerous human cases infested with the trophozoites and cysts of *Endolimax nana.* It was prepared in a variety of ways, including fixation in Schaudinn's fluid plus 5 per cent and 20 per cent glacial acetic acid. The staining was done entirely with Heidenhain's hematoxylin.

## OBSERVATIONS

Study of the trophozoites of *Endolimax nana* reveals that when they are fixed in Schaudinn's plus 5 per cent glacial acetic acid, the following nuclear picture is produced. The karyosome is large, homogeneous and stains very intensely with hematoxylin, being one of the last elements to lose its stain on differentiation. Spoke-radii connect it with a faint nuclear membrane, on which the only apparent chromatin is arranged as small lumps at the distal ends of these radii (Text fig., 1 and 2). However, when 20 per cent glacial acetic acid is added to the fixative, a marked difference from the above is noticed. The karyosome

now stains lightly and rapidly loses color with even moderate exposure to differentiation. The radii are present, but the nuclear membrane is now readily seen to be encrusted with a uniform ring of small granules (4 and 5).

In the cysts, the same results are obtained, but, due to the general reduction in amount of nuclear chromatin on encystment in *Endolimax nana*, the effects of the 20 per cent acetic are more difficult to observe. In Schaudinn's plus 5 per cent acetic, the karyosomes are dark, but the radii and distal chromatin lumps are usually not prominent (3), while in Schaudinn's plus 20 per cent acetic the karyosomes are again faint and careful observation reveals a series of chromatin lumps on the periphery of the nucleus (6).




Text Figure.—Camera lucida drawings of *Endomilax nana*. Description in text. × 3,000.

These observations were confirmed many times. Smears from the same small bit of feces, treated identically save for the difference in acetic acid content of the fixative, yielded the results pictured in the text figure, where numbers 1, 2, 4 and 5 are from slides made from the same small fecal mass. The cysts, numbers 3 and 6, are from another case, similarly treated.

## DISCUSSION

The early descriptions of what is now known as *Endolimax nana*, by Gauducheau (1908), Elmassian (1909), Wenyon (1912, 1913, 1915 and 1916), Chatton and Lalung-Bonnaire (1912), James (1914) and others, agree in that the organism was not regarded as a true parasite of man. The nucleus was described as having a large karyosome, a very faint nuclear membrane, on which there was little or no peripheral chromatin.

Since the true status of the amoeba has been recognized, the works of Swellengrebel and Winoto (1917), Wenyon and O'Connor (1917), Dobell and Jepps (1917), Kuenen and Swellengrebel (1917), Brug (1918), Dobell (1919) and Wenyon (1926) have added little to the early conception of the nuclear structure, except for the observation of the spoke-radii and the small lumps of chromatin at the distal ends of these radii. Dobell (1919) states that the nucleus has "a well-marked nuclear membrane in which minute granules—possibly of chromatin—can sometimes be seen" and that he has not been able to convince himself "of the existence of any 'peripheral chromatin' in the clear zone" between the nuclear membrane and the karyosome. Wenyon (1926) asserts that there "is a definite nuclear membrane which appears to be free from chromatin, all of which seems to be concentrated in the karyosome."

The present study shows the existence of an abundance of this so-called peripheral chromatin in the trophozoites of *Endolimax nana,* the proper technique only being necessary to demonstrate it clearly. This is in agreement with some of the results on the *limax*-amoebae from other hosts. Mackinnon (1914) described peripheral chromatin in *Vahlkampfia* sp. from the Crane-fly larva. Lucas (1927) saw peripheral chromatin in one specimen of *Endolimax blattae* from the cockroach. In *Endolimax termitis* from *Mirotermes hispaniolae* Kirby (1927) records an abundance of this chromatin. Hogue (1915), though failing to find chromatin on the nuclear membrane in *Vahlkampfia calkensi* from oysters, did note it (Hogue 1921) in *Vahlkampfia patuxent,* also from the oyster. Others, Dobell (1914) in *Amoeba lacertae* from *Lacerta muralis,* Tyzzer (1920) in *Pygolimax gregariniformis* from the domestic fowl and turkey, Chiang (1925) in *Endolimax ratti* from laboratory rats, make no mention of this chromatin, or state definitely that none is present.

These references do not represent a complete list, but suffice to show the varied observations on the nuclear structure of the *limax*-amoebae. The suggestion presents itself that, as in the case of *Endolimax nana* where no true peripheral chromatin was stated to be present, a variety of techniques would be most desirable in further studies on these amoebae and they may be found to be more similar morphologically than previously described.

In conclusion, the results herein recorded show a closer relationship between *Endolimax nana* and the species of Entamoeba, in which peripheral chromatin is characteristic. Definite physical or chemical differences are suggested by the differential behavior of the karyosome and peripheral chromatin of *Endolimax nana* to the change in the acetic acid content of the fixative. These two elements do not react

alike and therefore must be different. The nature of this difference is still to be determined.

SUMMARY

1. The typical picture of *E. nana* is produced by fixing in Schaudinn's fluid plus 5 per cent glacial acetic acid; Schaudinn's fluid plus 20 per cent glacial acetic acid produces notable differences: The karyosome retains hematoxylin poorly; A definite ring of peripheral chromatin appears on the nuclear membrane.

2. A difference in composition is indicated between the karyosomal and peripheral chromatin.

3. The presence of the peripheral granules makes Endolimax a close relative of Entamoeba.

4. The use of a variety of techniques is suggested for the study of these amoebae.

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Other references cited will be found either in Dobell or Wenyon.

# A COMPARATIVE STUDY OF THE MALE TERMINALIA OF CALIFORNIAN ANOPHELINES

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The terminal segments of the mosquitoes were clipped from anesthetized specimens and placed in a watchglass containing a bit of cotton soaked in 30 per cent alcohol. They were immediately mounted on slides in Gater's medium and were allowed to clear for forty-eight hours or more before they were examined. Camera lucida drawings were then made of the terminalia which are diagrammatic to the extent that they are bilaterally symmetrical, whereas, due to difficulties in mounting, only a few of the mounted specimens show this symmetry. The processes of the ninth tergite and the anal lobe have been omitted from the drawings as they are apt to obscure the other structures of greater taxonomic importance.

*Anopheles pseudopunctipennis* THEOBALD [TEXT FIGURE A]

Side-piece, almost twice as long as width at base.

Internal spine, prominent, about half-way distad of the middle of the side-piece.

Parabasal spines, two, the inner spine not as long as the outer spine and recurved at tip, both spines taper to a point.

Clasper, longer than side-piece and slightly constricted in the middle, apical claw short.

Claspettes, bilobed, the ventral lobe dome-shaped, with two stout tapering setae at apex; the dorsal lobe elongate with three strong setae.

Mesosome, cylindrical with a slight bulge at apex, apical opening without leaflets. No specimen of our species of *pseudopunctipennis* yet examined has had the "four, delicate, serrate 'leaflets' " noted by Root (1924) on specimens from Mexico.

*A. maculipennis* MEIGEN [TEXT FIGURE B]

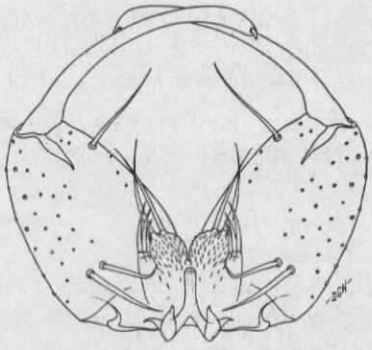
Side-piece, at least one and one-half times as long as wide.

Internal spine, slightly distad of middle.

Parabasal spines, two, stout; outer spine long, tapering to a point; inner spine broad, recurved at tip.

Claspers, longer than side-piece, constricted medianly.

Claspettes, bilobed, the dorsal lobe small with two pointed spines, the ventral lobe larger with two or three spines, usually the latter.



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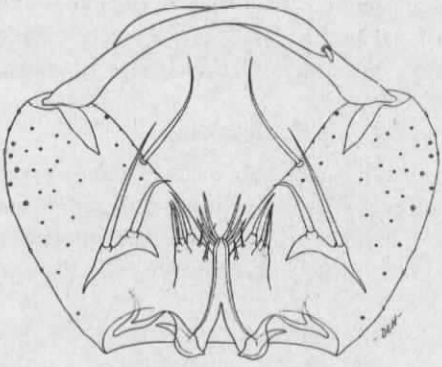

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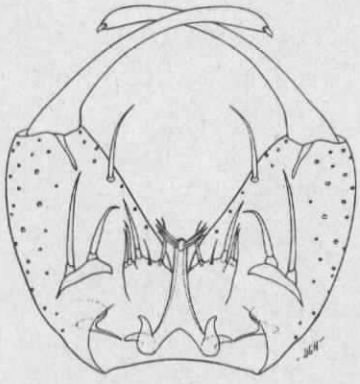

C

Mesosome, stout, cylindrical, with three pairs of apical leaflets, the pairs of leaflets becoming progressively longer posteriorly.

*A. punctipennis* SAY [TEXT FIGURE C]

Side-piece, nearly twice as long as wide, conical.

Internal spine, prominent, arising one-third from the apex of the side-piece.

Parabasal spines, two, short, stout, arising from a prominent tubercle; the inner spine recurved at tip.

Clasper, longer than side-piece, apical claw short, stout.

Claspettes, bilobed, dorsal lobe small, with two closely set, short, stout spines; ventral lobe much broader with an outer large, broad and sharp-pointed spine, an inner smaller spine and midway between these spines a hair.

Mesosome, more slender than that of *maculipennis* but longer and stouter than that of *pseudopunctipennis;* apically three pairs of approximately equal leaflets, more slender than those of *maculipennis.*

## CONCLUSIONS

The terminalia of our Californian Anophelines may be used for taxonomic purposes. The salient points of differentiation are: the length of the side-pieces, the point of origin of the internal spine, the leaflets of the mesosome and, most important of all, the morphology of the claspettes

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# *TRICHOMONAS PHASIANI*, A NEW FLAGELLATE FROM THE RING-NECKED PHEASANT, *PHASIANUS TORQUATUS* GMELIN*

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An examination of fecal material from the cecum of a female pheasant revealed the presence of myriads of trichomonad flagellates. Intestinal smears were fixed in Schaudinn's fluid and were stained in Heidenhain's iron-hematoxylin. In 1929 Tyzzer published a paper on the Coccidia of gallinaceous birds and described *Eimeria phasiani*, but there seems to be no record of a flagellate from this host. Donné in 1937 established the genus *Trichomonas* with *T. vaginalis* as the genotype. He did not accurately determine the number of flagella, but Künstler in 1884 and Lynch in 1915 state that there are four anterior flagella in this species. Kofoid (1920) retains *Trichomonas* for those trichomonads possessing four anterior flagella.

The trichomonads described from birds are: *Trichomonas columbae* Rivolta (1878) from the pigeon, *T. gallinarum* Martin and Robertson 1911 from the chicken, *T. anatis* Kotlan 1923 from the duck, *T. oti* Tanabe 1926 from the screech owl, *T. avium* Cunha and Muniz 1926 and *T. lanceolata* Cunha and Muniz 1926 from Brazilian birds, *T. flordinae* Hegner 1929 and *T. ortyxis* Hegner 1929 from the valley quail, *T. anseri* Hegner 1929 from the goose, *T. diversa* Volkmar 1930 from the turkey, *Tritrichomonas eberthi* Martin and Robertson 1910 from the chicken, and *T. bonasae* Tanabe 1926 from the grouse.

## *Trichomonas phasiani* N. SP.

This flagellate is slender pyriform with a body curvature varying according to the arcuation of the chromatic basal rod (Fig. 1). The anterior tip of the organism is obtusely rounded. The posterior end tapers to a point. The cytoplasm stains lightly with hematoxylin. A large cytostome is present on the ventral side near the origin of the flagella.

A single large blepharoplast is situated at the extreme anterior margin of the body. Martin and Robertson (1911) were able to distinguish four basal granules in *T. eberthi*, but only one can be demon-

* The writer is deeply indebted to Dr. E. R. Becker for his encouragement and help in the preparation of this paper.

strated in this species. Five flagella and a chromatic basal rod arise from the blepharoplast. Four of the flagella are anterior and subequal in length; two as long as, or slightly longer than the body; one of medium length slightly shorter than the long ones; one distinctly shorter than the one of medium length (Fig. 3). The fifth flagellum runs along the outer margin of a well developed undulating membrane and extends free caudally about half the length of the body. The chromatic basal rod is filiform, stains darkly with hematoxylin, and curves dorsally from the blepharoplast to the posterior end of the body, ending near the axostyle. The axostyle is slender, hyaline, and quite inconspicuous, projecting slightly and ending in a point. It stains so lightly with hematoxylin that it can seldom be traced to its origin, the blepharoplast.

The ellipsoidal nucleus located just behind the basal granule is almost indistinguishable, staining very lightly with hematoxylin. It is surrounded by a thin membrane and seems to be filled with minute chromatin granules. The karysome is small and may be either centrally (Fig. 1) or eccentrically (Fig. 2) placed with a definite clear area or achromatic capsule surrounding it. A few specimens are present with nuclei that stain an even gray, which may be caused by diffuse chromatin (Fig. 5). No cysts were observed.

Measurements of 500 individuals on stained slides are presented in the correlation table.

TABLE 1.—*Length and Width Correlation of Trophozoites of* Trichomonas phasiani, *n. sp.*

| Width in Microns | Length in Microns | | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5.5 | 6 | 6.5 | 7 | 7.5 | 8 | 8.5 | 9 | 9.5 | 10 | 10.5 | 11 | 11.5 | |
| 1.5 | 1 | .. | .. | 1 | .. | .. | 1 | .. | .. | .. | .. | .. | .. | 3 |
| 2.0 | 1 | .. | 2 | 3 | 14 | 3 | 8 | 2 | .. | .. | .. | .. | .. | 33 |
| 2.5 | .. | .. | 3 | 6 | 17 | 9 | 24 | 12 | 4 | 3 | 1 | .. | .. | 79 |
| 3.0 | .. | .. | .. | 18 | 37 | 26 | 69 | 50 | 12 | 22 | 8 | .. | 1 | 243 |
| 3.5 | .. | .. | .. | 4 | 12 | 6 | 24 | 20 | 8 | 13 | 2 | .. | .. | 89 |
| 4.0 | 1 | .. | .. | .. | 5 | 1 | 6 | 17 | 3 | 8 | 3 | .. | .. | 44 |
| 4.5 | .. | .. | .. | .. | .. | 1 | .. | 3 | 2 | 1 | 1 | .. | .. | 8 |
| 5.0 | .. | .. | .. | .. | .. | .. | 1 | .. | .. | .. | .. | .. | .. | ... |
| Total | 3 | | 5 | 32 | 85 | 46 | 133 | 104 | 29 | 47 | 15 | | 1 | 500 |

| | Length | Width |
|---|---|---|
| Range | 5.5–11.5μ | 1.5–5μ |
| Mean | 8.519μ | 3.051μ |
| Standard deviation | 0.943μ | 0.541μ |

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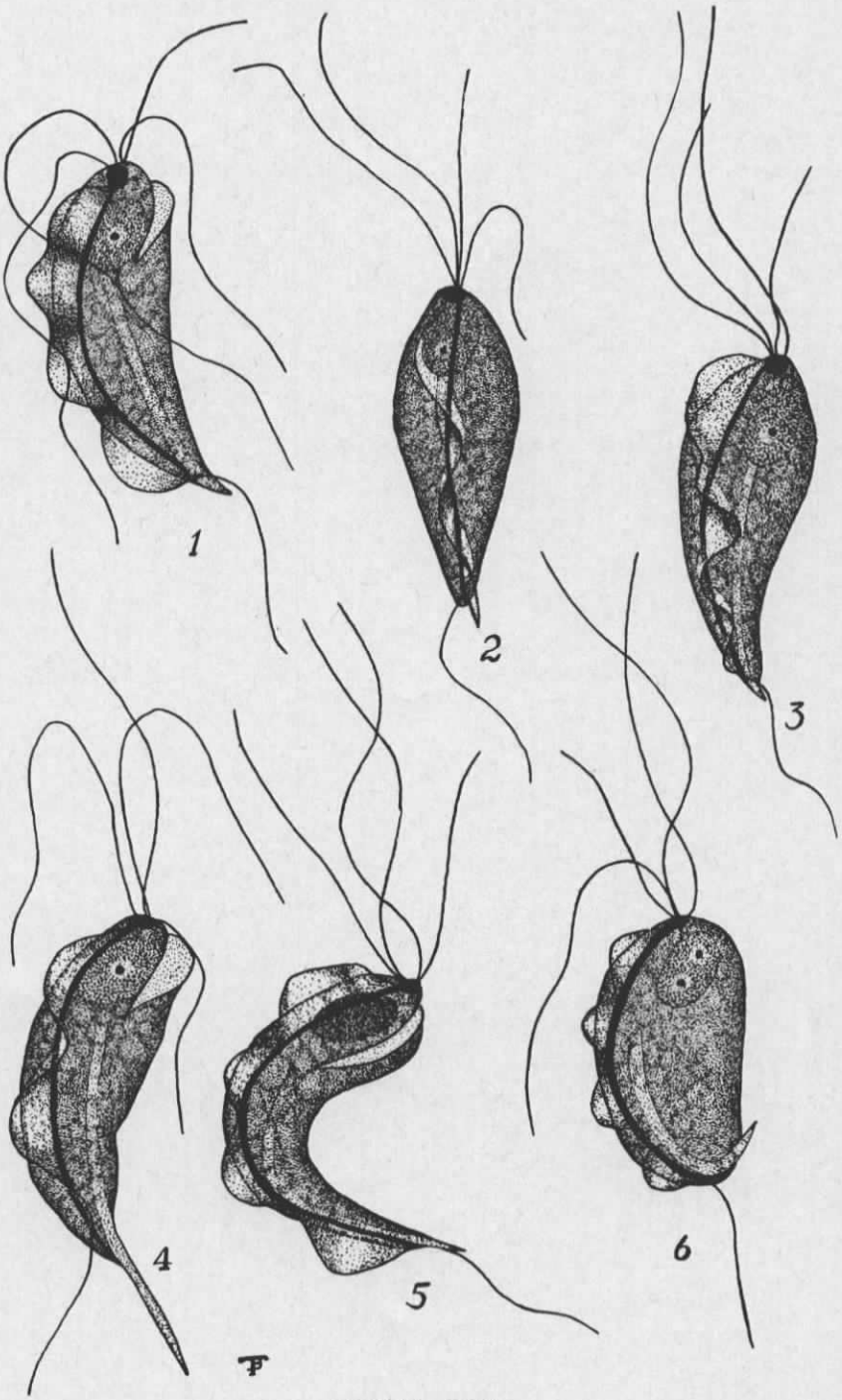


PLATE XXV

Martin, C. H., and Robertson, M. 1911.—Further Observations on the Caecal Parasites of Fowls with Some Reference to the Rectal Fauna of Other Vertebrates. Quar. Jour. Micr. Sci., 57:53-81.

Volkmar, F. 1930.—Trichomonas diversa n. sp. and its Association with a Disease of Turkeys. Jour. Parasit., 17:85-89.

Wenyon, C. M. 1926.—Protozoology. A Manual for Medical Men, Veterinarians and Zoologists. 2 vols. London.

Other references cited will be found in Wenyon.

EXPLANATION OF PLATE XXV

× 4,000

Fig. 1.—Typical trophozoite.

Fig. 2.—Dorsal view of a trophozoite.

Fig. 3.—Trophozoite illustrating flagellar lengths.

Fig. 4.—Trophozoite with long axostyle.

Fig. 5.—Trophozoite with a dark staining nucleus.

Fig. 6.—Trophozoite with distorted axostyle and two blepharoplasts.

# *PHYSALOPTERA POLYDENTATA* N. SP.*

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The material upon which this species is based consisted of male and female specimens taken from a Gecko (*Hemidactylus mabouia*) obtained in Tanganyika Territory, British East Africa, and placed at my disposal through the courtesy of Dr. H. A. Baylis of the British Museum (Natural History).

The cuticula shows a very delicate transverse striation and is partially reflexed over the lips of both sexes. The excretory pore opens about 10 to 20$\mu$ back of the cervical papillae which are located opposite the cephalic end of the posterior division of the esophagus. The two lateral lips are large and rounded in lateral view and are sharply set off from the body. Each lip carries a pair of large sub-median papillae set deeply in the greatly inflated cuticula. Each lip is provided with a large outer and a small inner median tooth and a pair of inner teeth at each lateral angle. These lateral teeth are but slightly differentiated in size and position from the very distinct dentigerous ridge of from five to six members which extends almost the entire distance from the inner tooth to the lateral pair.

The esophagus is straight, opens directly into the mouth, shows definitely an anterior glandular and a posterior muscular portion, and measures 1/7 of the total length of the female and 1/6 of that of the male. The nerve ring surrounds the posterior fifth of the anterior portion of the esophagus.

The number of uteri is very definitely four, which places this form in the group Tetradelphys as established by Ortlepp (1922). The vulva opens at about the level of the posterior end of the esophagus, usually slightly caudad of that position. From the vulva to the division of the common trunk into the two primary branches is about 1/9 of the body length i. e., 3.5 mm, and from the distal end of the common trunk to the secondary branches about 0.5 mm. The secondary branches of the uteri are extremely intertwined and difficult to follow to their respective ovaries.

The male bursa is long and pointed, with a slight ventral flexion. The under surface is ornamented by a cluster of pre-cloacal superficial disc-like markings which closely approximate in their position the pre-cloacal sessile papillae. The four pairs of stalked papillae are evenly spaced, the two middle pairs being slightly the longer. Two pairs are

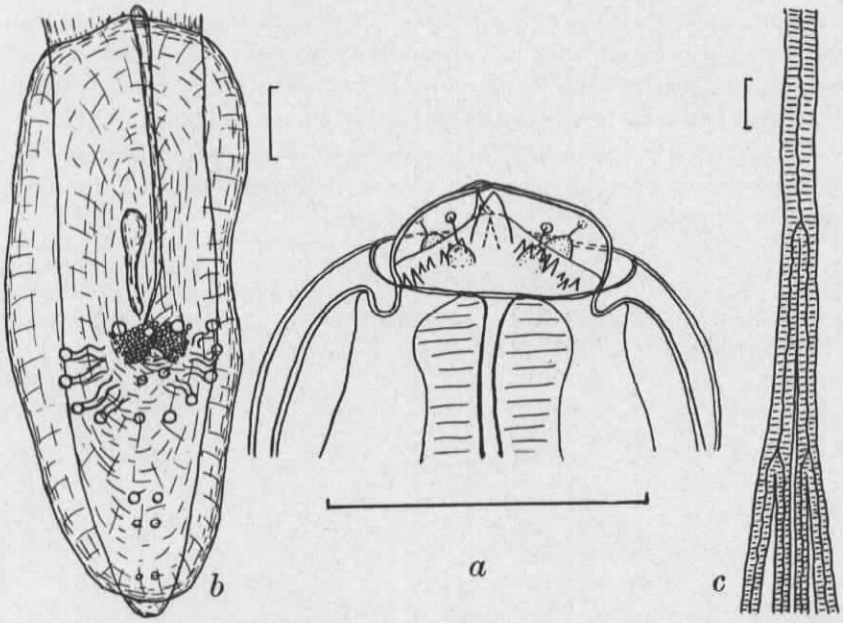
* Contribution from the Biological Laboratories of Knox College, No. 42.

pre-cloacal, and two pairs post-cloacal in position. The three pre-cloacal sessile papillae are of about equal size, the middle one being slightly nearer the cloacal lip. The post-cloacal papillae are in three groups; pairs 1 & 2, 3 & 4, and pair number 5. The first and last pairs are somewhat smaller than the others.

The spicules are very unequal, the left being long and slender while the right one is short and stout.

The detailed measurements are as follows:

*Male:* Body length, 12.65 mm.; greatest width, 0.4 mm.; length of anterior esophagus, 0.375 mm.; length of posterior esophagus, 1.9 mm.;




DESCRIPTION OF TEXT FIGURE

*Physaloptera polydentata* n. sp. *a*, anterior end of male; *b*, tail of male, ventral aspect; *c*, diagrammatic sketch of the division region of the ovarian ducts. Scale in all figures equals 0.1 mm.

head-cervical papillae distance, 0.39 mm.; head-excretory pore distance, 0.4 mm.; head-nerve ring distance, 0.25 mm.; cloaca-tail distance, 0.4 mm.; length of spicules, left—0.425 mm., and right—0.1625 mm.

*Female:* Body length, 30 mm.; width at vulva, 0.45 mm.; length of anterior esophagus, 0.425 mm.; length of posterior esophagus, 4 mm.; head-cervical papillae distance, 0.51 mm.; head-excretory pore distance, 0.52 mm.; head-nerve ring distance, 0.39 mm.; head-vulva distance, 3.5 mm.; anus-tail distance, 0.5 mm.; size of ova, 0.025 mm. by 0.05 mm.

*Habitat:* Intestine.

*Host: Hemidactylus mabouia* (Gecko).

*Range:* Tanganyika Territory, British East Africa.

*Type Specimens:* No. 10.23.138-142.1929. Helminthological Collections of the British Museum (Natural History).

This form, possessing as it does, the tetradelphys form of uteri and also dentigerous ridges, is closely related to *Physaloptera quadrovaria* Leiper (1908), *P. paradoxa* v. Linstow (1908), and *P. pallaryi* Seurat (1917). It is easily distinguished from these three species by the differences in the relative proportions of the various divisions of the uterine structures, by the plan and ornamentation of the male bursa, and by the number and arrangement of the denticles. This form resembles most closely *P. quadrovaria,* but even in this case the separation is easily made on the plan of subdivision of the common trunk of the oviduct; the primary divisions in *quadrovaria* being almost entirely eliminated while those of *polydentata* are of considerable length.

This form has been called *Physaloptera polydentata* n. sp. because of the presence of dentigerous ridges, a condition not of common occurrence in the tetradelphoid forms of this genus.

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# A NEW LARVAL CESTODE, PROBABLY *HYMENOLEPIS CUNEATA*, A TAPEWORM OF A WILD DUCK

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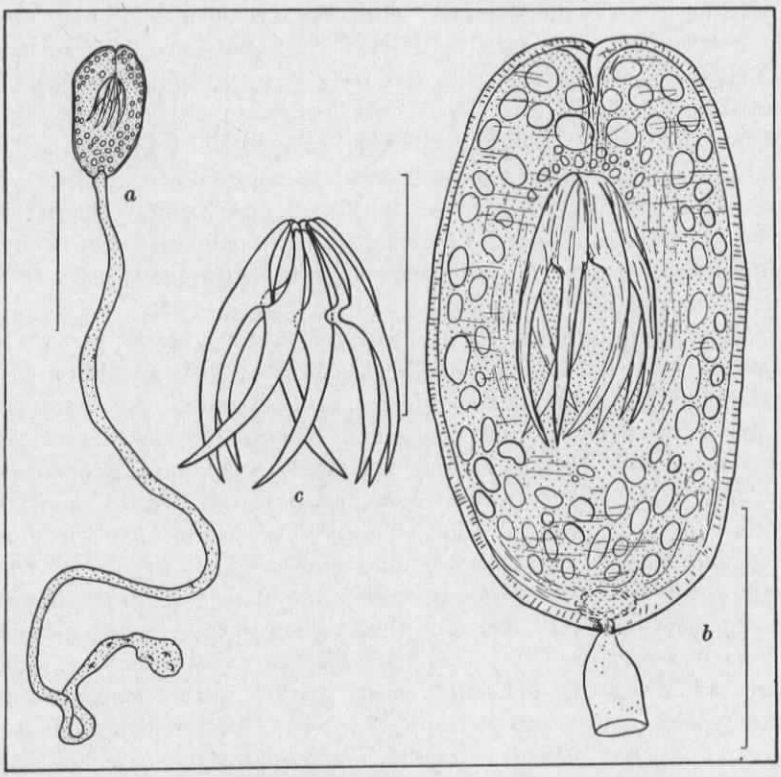
During the summer of 1927 while engaged in a field study on the life history and frequency of *Diphyllobothrium latum*, organized by Dr. Henry B. Ward and supported by a grant to him from the Committee on Medical Research of the American Medical Association (Ward, 1927, 1929), and in connection with another investigation I was making (Essex, 1927), a large number of copepods from Long Lake, Ely, Minnesota, were examined for larval tapeworms. Magath first called my attention to an interesting procercoid in the body cavity of *Diaptomus oregonensis*. This parasite was subsequently seen in two other copepods of this species.

When liberated from the body cavity of the host the parasite was markedly quiescent as compared to the great activity exhibited by the larvae of certain other species which I have observed. An examination of the larvae revealed that they were composed of two distinct body regions, the body proper and an exceedingly long caudal appendage or cercomer. The body portion which measured 0.23 by 0.13 mm., was ovate, and the anterior margin in the terminal region turned in toward the middle where a set of hooks could be distinguished (Figs. *a* and *b*). In the living specimen only four hooks could be seen distinctly, but after the organism had been allowed to disintegrate slightly eight hooks could be clearly seen (Fig. *c*). The hooks, which measured about 110$\mu$ in length and about 22$\mu$ in their broadest portion, were arranged roughly in four pairs. Acetabula were not observed. Scattered throughout the body portion were many refractile bodies of varying size and shape. Such bodies were not seen in the cercomer. This part of the organism measured about 1.8 mm. in length with an average diameter of about 0.03 mm. The terminal portion was somewhat broadened and bore the six cast-off hooks of the oncosphere.

## DISCUSSION

The hooks of parasitic worms are considered one of the most reliable diagnostic characters in the identification of species. When such structures are present in a species it is possible to identify the larval form with the adult parasite with a reasonable degree of accuracy. The character of the hooks of the larva just described indicates that the adult

form is not a tapeworm of fish. An inspection of the hooks immediately suggests the genus Hymenolepis. Since certain of the species belonging to this genus are harbored by aquatic birds a comparison of the hooks with those of the known avian species is the logical procedure. It is fortunate that an extensive study of this group has been published by Mayhew (1925). Due to his detailed record of the salient morphologic features of previously described and new American species it has not




TEXT FIGURE

Camera lucida tracings. *a*, procercoid immediately after removal from body cavity of *Diaptomus oregonensis*; *b*, body portion of procercoid much enlarged; *c*, hooks after organism had become slightly disintegrated. Scale in *a* equals 0.3 mm.; in *b* and *c*, 0.1 mm.

been a difficult task to refer the larva in question to the probably adult form. A study of the text and figures on the different species of Hymenolepis described by Mayhew revealed that only one species possessed hooks that answered the description of those borne by this larval cestode. On the basis of the close similarity of the hooks of the procercoid under consideration to those of *Hymenolepis cuneata*,

(Mayhew, 1925) it is reasonably safe to identify this larval tapeworm as the procercoid of a species of Hymenolepis probably *H. cuneata,* a tapeworm of a wild duck.

This report is being made in the hope that it may be of service to some future worker who may be interested in the life cycles of the tapeworms of aquatic birds.

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# PORCUPINE LOUSE INFESTING THE MONKEY*

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A *Macaque rhesus* monkey suffering from a severe dermatitis was brought to the Veterinary Department of the University of Minnesota from the Como Zoological Gardens of St. Paul on May 9, 1931. Upon examination an unusually heavy infestation of biting lice (Mallophaga) was found but no sucking lice, which are quite common on monkeys, were observed.

A collection of lice made from this animal consisting of 7 males, 7 females and 4 immature specimens after clearing the material has been identified as *Eutrichophilus setosus* (Giebel) Mjoberg, the porcupine louse. This is a common parasite on the Canada porcupine, *Erethizon dorsatum,* and has been reported from Lake and St. Louis Counties of Minnesota by Jellison (1931). A cage of porcupines was maintained in the gardens near the monkeys and is the probable source of this infestation. Six species of Mallophaga that have been found on various primates are recorded by Stiles (1929) but these were believed to be on their normal hosts. The degree of infestation, especially of immature specimens, found in this instance indicates that the porcupine louse was well established on its new host.

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