# I. A STUDY OF THE LIFE HISTORY OF *TRICHOBILHARZIA CAMERONI* SP. NOV. (FAMILY SCHISTOSOMATIDAE).

# II. A STUDY OF THE LIFE HISTORIES OF FOUR NEW SPECIES OF *PLAGIORCHIS* (FAMILY PLAGIORCHIDAE).

# III. A STUDY OF THE LIFE HISTORY OF *NOTOCOTYLUS STAGNICOLAE* HERBER (1942) (FAMILY NOTOCOTYLIDAE).

A Thesis

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CONTENTS

## SUMMARY

Six species of trematodes, of which five are regarded as new, have been investigated. All had cercarial stages in snails occurring in the Ottawa River.

1. Trichobilharzia cameroni sp. nov. is described. Its cercarial stage occurs in the snail Physa gyrina and is a cause of Swimmer's Itch locally. The adults and all stages in the life cycle are described in detail. Canaries, pigeons and domestic ducks were experimentally infected. Ducks, up to at least four months of age, could be infected and retained the infection and passed viable eggs for four months subsequently. Snails liberated carcariae for four to five months and most carcariae remained alive in water at room temperature for up to three days; a few lived longer.

2. Four species of Plagiorchis, all of which are regarded as new (e.g. Plagiorchis stagnicolae, P. palustris, P. canadensis, and P. ottawensis spp. nov.) are described from pigeons experimentally infected from metacercariae encysted in dragon-fly larvae; these in turn were infected with cercariae obtained from wild Stagnicola palustris. Cercariae, metacercariae, and adult flukes are described in detail and comparisons drawn with previously described species.

3. Cercariae of Notocotylus stagnicolae were also found in Stagnicola palustris and the life cycle was completed in chickens fed on metacercariae produced by encystment of the cercariae on the dish containing the snails. The various stages are described and attention is drawn to a number of slight differences between the present material and previous descriptions. The process of cyst formation by the metacercaria was studied in detail.

# PART I. A STUDY OF THE LIFE HISTORY OF

# *TRICHOBILHARZIA CAMERONI* SP. NOV.,

# (FAMILY SCHISTOSOMATIDAE)

## INTRODUCTION

Since the outbreak of swimmer's itch in the vicinity of Ste. Anne de Bellevue near Montreal, during the summer of 1949, investigations on this problem have been undertaken by members of the Institute of Parasitology. The dermatitis schistosome seems to be present sporadically along the Ottawa River in the vicinity of this town, because cases are frequently reported. A more thorough study of the same problem was made during the late fall and winter of 1951. The purpose of this study is to elucidate the biology of the parasites concerned in the hope that our understanding of them may suggest some means for their control. The local species was identified as belonging to the genus *Trichobilharzia*.

## HISTORICAL REVIEW

The genus *Trichobilharzia* belongs to the subfamily Bilharziellinae of the family Schistosomatidae, and was created by Skrjabin and Zakharow, 1920, for their species *T. kossarewi* from wild ducks in Russia. Brumpt, 1931, by experimental infection of domestic ducks with *Cercaria ocellata* from *Limnaea stagnalis*, subsequently recovered eggs and fragments of the adults from those ducks. He identified them as *T. kossarewi*; because of priority, he proposed that the name should become

Trichobilharzia ocellata (La Vallete, 1854). No measurements were given in his description. The gynecophoral fold was not shown in his drawing.

Oiso, 1927, described Bilharziella yokogawai from an experimental infection of ducks in Taiwan (Formosa). Because of the long and narrower posterior portion of its body (instead of a narrow anterior and a broader lanceolate posterior end), it was placed in the genus Pseudobilharziella by McLeod, 1937. Ejsmont (1929) had established this genus for the elongated filamentous male specimens described by Kowalewski (1859) as young males of Bilharzia polonica in Russia. Pseudobilharziella was created chiefly on the basis of the presence of the gynecophoral fold; Kowalewski's misidentified material was renamed P. kowalewskii.

McLeod (1937) described Pseudobilharziella querquedullae from Querquedula discors, based on the male specimens only. A few years later, he infected birds experimentally with Cercaria physellae Talbot, and completed the description for the female (1942). The eggs of McLeod's species are very similar to those of Bilharziella yokogawai, the chief difference between them lying in the strikingly forward position of the gynecophoral fold in the latter. P. querquedullae was later considered a synonym of Trichobilharzia *physellae* by McMullen and Beaver (1942).

Szidat (1938) described *Pseudobilharziella filiformis* from *Cygnus olor* L. The eggs are oval and carry a small spine at the broader end.

Yamaguti (1941) described a new species, *Pseudobilharziella corvi* from the intestinal and the mesenteric veins of *Corvus corone corone* Linne from Japan. It has a very long body but the most characteristic feature is that the vas deferens opens into the seminal vesicle about the middle of the latter.

McMullen and Beaver (1942) by experimental infection of birds with *Cercaria physellae* Talbot, 1936, *Cercaria stagnicolae* Talbot, 1936, and *Cercaria elvae* Miller, 1923, recovered the adults and eggs of three species of trematodes which they identified as *Trichobilharzia physellae* (Talbot), *T. stagnicolae* (Talbot), and *T. ocellata*. *T. ocellata* from *Cercaria elvae* was considered by these authors to be identical to that described by Brumpt, 1931, because of the similarity of the eggs produced.

They raised the question of the identity of the genus *Pseudobilharziella*. According to their opinion:

> "Ejsmont (1929) established the genus *Pseudobilharziella* largely on the basis of the presence of a gynecophoric canal, but a study of the genera in question indicates

> that this structure is present in both. Consequently *Pseudobilharziella* Ejsmont, 1929, becomes a synonym of *Trichobilarzia* Skrjabin and Zakharow, 1920."

Consequently, they proposed to transfer all the species so far described under the genus *Pseudobilharziella* to the genus *Trichobilarzia*.

## MATERIAL

Snails of the species *Physa gyrina* and *Stagnicola palustris* were collected from a beach of the Ottawa River by the middle of October, 1951. Swimmer's itch had been reported on this beach during the summer. Eleven snails of *Physa gyrina* were found giving off schistosome cercariae. Each snail was kept in a separate container. The cercariae which emerged were used for experimental infections. During the latter part of the study, cercariae from experimentally infected snails were used. The snails of the species *Physa gyrina* and *Stagnicola palustris* used for the study of the various stages of the life history, were laboratory-bred. *Limnaea stagnalis* (L.) *var.* was collected from a pond in a private garden where schistosome dermatitis has never been known.

The birds used for experimental infections were canaries, ducks, and pigeons. The young canaries and pigeons, one or two weeks old, were obtained from breeders. The ducklings, about two weeks old, came from Brome Lake Farm. All were kept under controlled conditions in the laboratory throughout the study.

## METHOD OF STUDY

### Technique

The eggs of the parasites were studied in a fresh condition in saline and water. They were preserved in Gilson's fluid which causes less shrinkage.

The droppings of the infected canaries were placed in seriological test tubes with well water. The miracidia thus hatched were removed for study and experimental infections. They were also obtained from a flask with a side arm (McMullen and Beaver, 1945) for the morphological study. The miracidia were studied unstained or stained with the vital stains: neutral red, methyl blue, nile blue, brilliant cresyl blue and Janus green. Methyl cellulose was also used to reduce the activity of the larvae. They were fixed in Bouin fixative and Gilson's fluid, for measurement.

The sporocysts were studied with fresh material, specimens fixed with Gilson's fluid and stained specimens. The specimens fixed in Gilson's fluid are transparent and clear and prove to be valuable for observation.

The preserved specimens were stained with borax carmine.

The cercariae from naturally infected snails were studied alive, unstained, or stained with vital stains. They were stained with very dilute neutral red and fixed in 10 per cent hot formalin (Talbot, 1936) for measurement.

The adults were obtained from the experimentally infected birds. The birds were injected with 1 per cent sodium citrate through the heart, soon after they were killed, to prevent blood coagulation. The parasites remained alive for five or six hours or even longer after the birds were dead. The number of parasites recovered from each bird was small. But this species is not too difficult to remove, as the parasites are in the peripheral blood vessels on the intestinal wall. When a worm was located, a break was made on the vessel about the middle of the worm and the worm could be squeezed out slowly by pressing it at both ends. The adults were frequently found in the liver. In this case, they were easier to remove. The liver was teased in the saline as soon as the birds were killed. After a couple of hours many adults were found in the saline or were partly exposed and could be dissected out with less difficulty. The morphology of the adults was also studied under fresh conditions, fixed in Gilson's fluid or stained. They were fixed in Gilson's fluid and Bouin fixative for sectioning. The preserved specimens were stained with Borax carmine and Harrison's haematoxylin.

Experimental Infection

The cercariae from the naturally infected snails collected were used for experimental infections. Approximately equal numbers of cercariae from each snail were used in order to ensure that both males and females were present. The birds to be infected were held in such a position that their feet were suspended in the vessel containing the cercariae. The time of exposure was usually from one-and-a-half hours to two hours. The infection was judged afterwards by the approximate number of cercariae left in the vessel and determination was made whether it should be reinfected.

The snails to be infected were placed singly in separate vials or small beakers. Usually three to six miracidia were added to each container. They were left together for a few hours before the snails were removed to the aquaria.

## LIFE HISTORY

The life history is similar to that of other schistosomes. The adults live in the veins on the intestinal wall and the bigger vessels supplying the intestine. They are also found in the liver. The eggs pass through the intestinal wall into the lumen and are voided with the faeces. In the water, the eggs liberate the miracidia which penetrate a suitable snail host. In the snail, each miracidium develops into a mother sporocyst which, in turn, gives rise to daughter sporocysts. The daughter sporocyst gives rise to the cercariae, which emerge into the water and penetrate the definitive host. The entire life history, from cercaria to cercaria, was completed experimentally in the laboratory. The details are considered below.

### Intermediate Host

Young laboratory-bred *Physa gyrina* Say, were used for experimental infections. When the snails and miracidia were placed together, the latter disappeared within half-an-hour. The percentage of infection was very high. Most of the snails that remained alive about one month after infection were found infected. These snails lived at most for a few weeks after the cercariae began to emerge. One naturally infected *Physa gyrina* remained alive for five-and-a-half months after being collected by the middle of October. From this snail numerous cercariae emerged every day. One hundred and thirty-eight cercariae emerged overnight after the snail had been kept for four-and-a-half months, and 43 cercariae after five months and six days. The experimentally infected snails were smaller in size and only a small number of cercariae emerged each day. Many attempts were made to infect laboratory-bred *Stagnicola palustris* and a few *Limnaea stagnalis* (L.) *var.* collected, and some of these snails were exposed to as many as 20 miracida each, but the results were negative. However, a conclusive statement should only be made after more experimental infections have been made. These snails were identified by Professor John Oughton of the Ontario Agricultural College.

Experimental Definitive Host

The natural definitive hosts of this species are not yet known. They are more likely to be migratory ducks than other local birds, because the schistosome cercariae have not been found from *Physa gyrina* which are numerous in a pond in a private garden which migratory ducks do not frequent.

Canaries are very convenient for obtaining material for study, but are quite sensitive to the parasites and the infections are reasonably low. They were usually exposed to from 100 to 150 cercariae and reinfected two or three days later if no symptoms appeared. A heavy infection usually resulted in pleural symptoms when the young parasites passed through the lungs and caused the death of the birds. Fourteen canaries were used in the experimental infections. Three canaries, each exposed to from several hundred to about a thousand cercariae, became very sick the day after infection and were found dead two or three days later. The symptoms were chiefly of a pleural nature and included laborious respiration. On post-mortem examination, young flukes were found in the lungs, kidneys, livers and the mesenteric blood vessels as early as twenty-four hours after infection. Those young flukes had dark brown granules scattered between the oral and the ventral suckers. These granules are the disintegrated remains of the pigmented granules from the eye spots of the cercaria. Other canaries were exposed to between 100 and 150 cercariae and were reinfected two or three days later if no symptoms appeared. Some were infected several times at two or three day intervals with a smaller number of cercariae each time. Among those, five, with doses from 200 to 400 cercariae, died, in from a few days to about a week. Many of the remainder became crippled but recovered later. Others showed no symptoms after infection and only a few or no miracidia were recovered when the faeces were examined.

One canary was exposed to 110 cercariae and again to 165 cercariae two weeks later. It was killed 57 days after the last infection. Many eggs were present in the intestinal wall. Six adult females were found in the veins of the wall of the small intestine near the duodenum and one female in the wall of the rectum. Five females and 29 males were found in the liver. The worms found in the liver were slightly smaller than those found in the intestine. Eggs were also found in the liver and the miracidia were eventually recovered from them.

One canary was exposed to 195 cercariae and became seriously crippled but later recovered. It was killed 28 days after infection. Four females were found in the wall of the rectum and 18 females and 28 males together with eggs were found in the liver. Other organs were not examined.

Only three canaries appeared to be more

tolerant to the infection, the eggs passed being moderate in number. These were regularly reinfected for three to four months in order to supply eggs for other studies. Two of these birds were observed to pass eggs 12 to 14 days after infection. In one of these cases the passage of eggs reached its maximum about one month after infection, and remained at this level for about three weeks. Then egg production gradually decreased, only a few miracidia being recovered at the end of two months. The bird was then killed and only two females and four males were found in the intestine. A few females and males were found in the liver. On no occasion were both males and females found together in the vein on the intestinal wall.

Nine ducklings, about two to three weeks old, were exposed to the cercariae. One, exposed to about 500 cercariae, was found dead in about four weeks. A small number of eggs was found in the wall of the large intestine and a few in the small intestine. One bird was exposed to several thousand cercariae and was found dead four days later. There were numerous white spots in the lungs and many young flukes were found there. Three ducklings were exposed to 900, 2,000 to 3,000, and several thousand cercariae, respectively. All became sick, the latter two being crippled and very sick, before they were killed accidentally two months after infection. A few worms were found in the veins leading to the caecal region in each case; other organs were not examined. One duckling, exposed to about 3,000 cercariae, became very sick six weeks after infection. A moderate number of eggs and two worms were found in the wall of its large intestine. One duckling, exposed to about 5,000 cercariae, was found crippled 27 days after infection; it was very sick, refusing food, and was killed. A small number of males and females was found in the veins along the intestine and the caeca. Males were found chiefly in the large vein near the caecal region. Numerous eggs were found in patches in the intestinal wall as well as in the lumen of the intestine. Part of the intestine was kept in a temperature of $3^{o}$ or $4^{o}$C. for 36 hours. The miracidia were recovered later. Part of the intestine was kept at the same temperature for two weeks and the miracidia were subsequently recovered. One young duck, about one month old, was exposed to about 1,000 cercariae by foot penetration and to several thousand by feeding with water. It was killed seven weeks later. The result was negative. One duck, about four months old, was exposed to 2,032 cercariae from laboratory-bred infected snails on the 31st January. The cercariae had been kept for from 60 to 72 hours at room temperature after emerging from the snails and were rather sluggish; however, no cercariae were left in the vessel after exposure. The duckling was killed on the 16th March, about six weeks after infection. A small number of small patches of eggs and one female were found in the intestinal wall. Only the posterior half of the intestine was examined. Two ducks, about three-and-a-half months old, were exposed to about 1,500 and 3,460 cercariae on the 21st and 27th January, respectively. Only a few cercariae were left in the vessel after the exposure. The last duck was crippled for about a week but recovered. On the 11th May, their droppings were examined for miracidia. The first sample examined was negative, but 8 miracidia from three droppings collected within one hour, were found from the more heavily infected duck. One miracidium was again found on the 25th May, four months after infection.

It appears that young ducks are more sensitive to the parasites than are older ducks and obtain heavier infections. Ducks up to at least four months old become infected with the parasites which in due course become mature.

Four pigeons were used for experimental infections. A three-week-old pigeon was exposed to about 1,000 cercariae but about half of them were left in the vessel. The bird was killed five months after infection. Three males, but no females, were found in the intestine wall and several males in the liver. A smaller number of eggs was found in the wall of the large intestine near the cloaca, but were dead. A small number of eggs was found in the wall of the small intestine and in the lumen, and miracidia were recovered from intestinal scrapings and contents.

A two-month-old pigeon was exposed to about 2,000 cercariae. One wing became paralyzed and the bird died six days after infection. It was examined some hours later. No parasites were found in the blood vessels supplying the intestinal wall but a small number of young flukes was found in the lungs and liver. There was severe haemorrhage in the lungs.

A four-month-old pigeon was exposed to 900, 700, and 550 cercariae at one-week intervals. It was killed two months after the last infection but no parasites or eggs were recovered from the intestinal wall.

A six-month-old pigeon was exposed to 866, 700 and 600 cercariae at intervals within 18 days. It was killed two months after the last exposure. The result was also negative. Both of these pigeons were healthy and normal.

It appears that young pigeons will take the infection but will gradually lose it. The old pigeons are resistant to the infection, although the number of experimental birds used is not extensive. Apparently, pigeons are not epidemiologically important.

Adults

The adults are long, slender and delicate worms. The body is slightly flattened at the anterior end and appears to be oval in cross-section. In some specimens they are greatly flattened in front of the gynecophoral fold in the males and in the corresponding region in the females. The suckers are fairly well developed. The oral sucker is terminal. Its dorsal wall is thicker than the ventral wall and the opening is subterminal. The ventral sucker is solid and thickly set with spines. It has never been seen retracted. The cuticle is apparently smooth and no spines are definitely seen. The fine striations are often present on the anterior end. In some females the cuticle is somewhat tuberculated. The posterior end in both sexes is flattened and expanded into a somewhat trilobed spatulate structure. The oesophagus is simple and very long. It is greatly enlarged a short distance before the acetabulum after which it bifurcates. These branches reunited at about the same level in both sexes, at the posterior end of the reproductive glands. The caecum passes through the rest of the body in a zigzag course in the semi-contracted specimen, and ends a short distance from or very close to the posterior extremity. The excretory pore is terminal. The excretory bladder is a very small, subspherical sac lying close to the posterior extremity and discharges to the exterior through a short canal.

Male: Measurements of 23 moderately to well extended specimens (Fig. 2) from canaries, pigeons and ducks vary from 3.18 to 5.71 mm. long, the longest being from the duck. The greatest width is at the gynecophoral fold which cannot be measured as the margins are always inrolled on themselves. It is a well-developed fold and thickly set with spines on the ventral surface (Fig. 5). In one specimen the spines were seen on both ventral and dorsal surfaces. The long axis of the fold is usually arched. The measurement along the median line varies from 0.212 to 0.345 mm. When it is contracted, it is much shorter and broader (Fig. 7).

The width just behind the acetabulum (measured from 12 specimens) varies from 0.063 to 0.097 mm.

The oral suckers from eight specimens vary from 0.031 to 0.040 mm. in width by 0.038 to 0.047 mm. in length. The ventral sucker is greater in cross-diameter. The measurement of the ventral suckers, shown more or less in lateral view from seven specimens, varies from 0.054 to 0.064 mm., and a measurement of five ventral suckers shown in front view varies from 0.031 to 0.049 mm. by 0.045 to 0.051 mm. The ventral sucker is closer to the oral sucker than to the gynecophoral fold, although in some specimens it is about midway between them.

The intestinal bifurcation is at a short distance in front of the ventral sucker, and the caecal reunion is about the level of the prostate gland. In one moderately extended living specimen, in which the digestive tract was full of red blood cells flowing back and forth, the union was seen just at the posterior margin of the prostate gland. The caecum passes backward, looping between the testes in contracted specimens and lying straight on one side of the body in well extended specimens.

The testes are numerous. In nine specimens examined the number varies from about 80 to 110. They are arranged in a single row (Fig. 3), beginning immediately behind the gynecophoral fold and ending a short distance before the tip of the intestinal caecum (Fig. 8). They are tandem in well extended specimens and irregularly alternated on either side of the intestinal caecum in the contracted specimens (Figs. 4 and 6 ). Their shapes are spherical or sub-spherical or oval. A measurement of some random testes from the extended specimens varies from 0.020 to 0.026 mm. in diameter and from 0.017 to 0.031 mm. by 0.020 to 0.041 mm. Those near the posterior extremity are much smaller. The vas deferens passes forward from the posterior end and discharges into the anterior end of the seminal vesicle. The seminal vesicle is a convoluted tube making several coils as it passes backward. It is divided into two portions by a constriction, the anterior portion staining deeper. The seminal vesicle extends from a short distance behind the ventral sucker to a short distance in front of the gynecophoral fold. In some specimens its anterior border is just behind the acetabulum, depending on the contraction of the body. It leads into a well developed prostate gland. This, in turn, leads by a narrow ejaculatory duct, into a sac-like cirrus, which opens through a genital papilla on the right margin of the gynecophoral fold (Fig. 5). The prostate gland and the posterior end of the seminal vesicle were seen to be enclosed in a sac.

The distance between the oral sucker and the acetabulum varies from 0.226 to 0.370 mm., and the distance between the acetabulum and the gynecophoral fold varies from 0.230 to 0.536 mm.

Female: The females (Fig. 1) are somewhat shorter and narrower. A measurement of six moderately to well extended specimens from canaries varies from 3.82 to 4.94 mm. The greatest width is at the region of the ovary and the seminal receptacle. It is from 0.052 to 0.059 mm.

The oral suckers from seven specimens, vary from 0.025 to 0.034 mm. wide and 0.032 to 0.40 mm. long. The acetabula measured from 13 specimens vary from 0.030 to 0.047 mm. wide and 0.025 to 0.038 mm. long.

The distance between the anterior end and the acetabulum is from 0.213 to 0.342 mm. and the distance between the acetabulum and the ovary is from 0.179 to 0.250 mm. The intestinal bifürcation is at a short distance in front of the ventral sucker. . The caecal union was seen about the equator of the seminal receptacle. The intestinal caecum passes through the long posterior end of the body in a zigzag course and ends near the posterior extremity, as in the male.

The ovary is tubular, stout and makes several loops. Its length in seven specimens, varies from 0.016 to 0.033 mm. by 0.287 to 0.358 mm. It becomes narrower at the posterior end where it leads into the oviduct. The oviduct is a moderately long stout duct, which expands into a convoluted, sac-like seminal receptacle. The length of the seminal receptacle (from five specimens) varies from 0.018 to 0.042 mm. by 0.072 to 0.125 mm. It leads, at its posterior end into the uterus which bends sharply forward and opens through the genital opening immediately behind the acetabulum. The female genital opening was seldom seen. On one occasion, however, it was seen in front view and it appears to have a thick rim. The uterus is a convoluted tube. The oötype has never been observed. There is never more than a single uterine egg. Laurer's canal is a small coiled duct, discharging through an opening on a prominent papilla on the dorsal surface of the body. Its connection with other parts of the reproductive system was not observed. The vitellaria extend from just behind the seminal receptacle to the posterior end of the body, a short distance in front of the tip of the caecum. They are usually in groups of two or three, arranged irregularly, alternating with the intestinal loops (Fig. 9). The single vitelline duct, a fairly large duct, passes forward ventral to the seminal receptacle and the ovary, and it appears to join the uterus just in front of the ovary.

Egg: The egg is spindle-shaped (Figs. 10a, 11, 12) with a thin colourless shell. The axis is usually straight but sometimes may be slightly curved or with other variations (Fig. 13). It gradually tapers at both ends giving rise to a fairly long pole ending with a blunt tip, and a shorter, conical opposite pole carrying a short projection which is usually bent like a hook. Measurements of 35 eggs from canaries with living miracidia inside, examined in saline, vary from 0.147 by 0.057 mm. to 0.212 by 0.063 mm., the greatest length, and 0.181 mm. by 0.073 mm., the greatest width. Measurement of another 35 eggs in water varies from 0.066 by 0.198 mm. to 0.080 by 0.24 mm., the greatest width, and 0.188 by 0.070 mm. to 0.253 mm. by 0.077 mm., the greatest length. The larger size of the egg in water is due to the absorption of water (Figs. 10b to 10e). The anterior end of the miracidium may be toward either pole of the egg. Between the miracidium and the shell, there is a layer of thick, semi-solid, greenish substance. Vestigal protein and excretory granules are also found.

Miracidium: The miracidium (Fig. 14) appears to be cylindrical with two slight constrictions. It rotates with a spiral movement as it moves rapidly in the water. The anterior end tapers abruptly to a conical papilla. The body is covered with long cilia which do not cover the entire body surface as is the case in the miracidia of other schistosomes. The cilia are in patches arranged in four transverse rows. This arrangement of cilia appears to correspond to the arrangement of the epidermal cells as observed in other schistosome miracidia. The shape and size of the epidermal cells were not studied in detail.

The miracidia, fixed in Bouin fixative and Gilson's fluid, are much contracted. The measurement of 12 less contracted specimens fixed in Bouin fixative, varied from 38 to 44 μ by 110 to 146 μ, and 12 less contracted specimens in Gilson's fluid, from 38 to 45 μ by 112 to 129 μ. The size of a few living specimens, stained with very dilute neutral red or in methyl cellulose and under a cover glass, is from 175 to 190 μ by 35 to 42 μ.

<u>Excretory System</u>: There are two pairs of flame cells, one located lateral to the penetration glands and one about one-third the body length from the posterior end. The flame cells on each side are connected with two long collecting tubules. The anterior collecting tubule after making a mass of loose loops just behind the penetration gland passes backward and unites with the posterior collecting tubule, which also makes a mass of loops, into a short common collecting tube which opens laterally to the exterior at about one-fourth the body length from the posterior end.

"<u>Primitive Gut</u>:" It is a flask-shaped gland and coarsely granulated. It stains with basic dyes. Its posterior margin is limited by the lateral secretory glands. It opens at the tip of the anterior papilla.

<u>Penetration glands</u>: These are two large glands opening by ducts on each side of the base of the anterior papilla. They occupy about two-fifths of the body length at the anterior end. They are homogeneous in appearance and each has a single nucleus. They are not stained with either basic or acid dyes.

Lateral Secretory Glands: These consist of two clusters of unicellular glands. They are similar to those of the human schistosomes (Faust, 1924). Their openings were not determined but must be associated with the two lateral processes. They also, do not take stains. The lateral processes are the two prominent lateral tubular processes one on each side of the body just behind the first row of cilia. In this species, the penetration glands are found posterior to the lateral secretory glands, a condition differing from that shown by Brumpt (1931) for *T. ocellata*.

Germ Cells: The germ cells occupy the major portion of the posterior half of the body. They are more or less hexagonal and closely fitted together. They appear to be contained in an elongated, elastic sac, because it retains its sac-like structure, even when its shape changes during contraction and expansion of the miracidium. Each germ cell contains a large nucleus. The arrangement of the germ cells in this species differs from that in *Schistosomatium douthitti* (Price, H.F., 1931). According to her description, the germ cells are attached to the body wall by fibre-like extensions of the body.

Hatching: If the eggs are put into water, hatching usually takes place in from two to five hours, although some take as long as eight hours. Under the microscope, hatching has been observed to occur in about 15 minutes, the entire process being observed. An egg from droppings of a canary was removed in saline and was observed under a cover glass. The egg measured 70 by 191 μ in saline (Fig. 10a). The miracidium was usually motionless in saline although it may occasionally show laborious movement, usually limited to the anterior end. Then the saline was replaced by water. After twenty minutes the egg measured 77 by 209 μ (Fig. 10b). A narrow space appeared between the miracidium and the semi-fluid substance. The miracidium began to show constant movement, but no ciliary vibrations were seen. After half-an-hour it measured 90 by 226 μ (Fig. 10c). The space between the miracidium and the shell gradually increased, indicating that more water was absorbed. Then ciliary action began. In 37 minutes it measured 94 by 233 μ and the miracidium was moving actively (Fig. 10d). In 43 minutes, the size of the egg was greatly increased and the miracidium was moving vigorously. Before the size could be determined, the egg shell ruptured and the miracidium escaped. The splitting of the egg shell in all cases observed occurred at the middle third of the egg.

Longevity in Water at Room Temperature: The miracidia were obtained by test-tube method. Each miracidium was removed to a separate serological test tube as soon as it hatched, usually in not more than half-an-hour. Observations were made at one-hour intervals until midnight and then again early the next morning. Of about 125 miracidia observed, the majority was found to live from two to 13 hours. Some remained alive up to 17 or 18 hours, one even up to 21 hours, but they were inactive and were found near the bottom of the test tube. The penetration power of the miracidium appears to be limited to a rather short time. Apparently the power gradually decreases two or three hours after hatching.

First Generation Sporocyst: The mother sporocysts (Fig.15) are tubular and very slender, much longer than the daughter sporocysts. They are colourless and transparent. The body is covered with a thin layer of cuticle which is lined with a layer of protoplasm. In this protoplasmic layer many germ cells are embedded, each with a large nucleus. Two mature sporocysts with migrating daughter sporocysts, from the liver of a Physa, 12 days after infection, measured 3.84 by 0.12 mm. and 4.25 by 0.125 mm., respectively. The birth pore was not seen. Most likely, it is at the anterior tip as is the case in the daughter sporocyst. The lumen contains motile daughter sporocysts, germ cells and germ balls in different stages of development.

A small number of daughter sporocysts were found in a snail eight days after infection. Possibly the mother sporocysts may become mature as early as about a week. They were found in the liver, the margin of the mantle and the margin of the foot. Sometimes they were found attached to the viscera. On many occasions they were not found at all.

The spines at the anterior end of the daughter sporocysts are visible. These migrating daughter sporocysts with the spines serve as a means of differentiating the mother sporocysts from the daughter sporocysts that contain cercarial embryos with tails not yet developed.

Second Generation Sporocyst: The daughter sporocysts have the same appearance as the mother sporocysts: tubular and colourless. They are much shorter than the mother sporocysts. The young daughter sporocysts (Fig. 16) bear many stiff spines near the anterior end. The birth pore is terminal at the anterior extremity and communicates with the body cavity by a short narrow birth canal. The body wall is similar to that found in the mother sporocyst. It is covered with a very thin layer of cuticle which is lined with a reasonably thick protoplasmic layer, richly granulated. In this protoplasmic layer there are many germ cells embedded, each with a large nucleus. The lumen is fully packed with the germinal materials, except the very anterior portion. The young sporocysts are reasonably active. They show active contraction and expansion, especially the anterior end, with the germinal materials in the lumen flowing back and forth. The spines are only present in the young forms. They disappear later. Cort (1943) suggested that probably these spines make migration possible. Some of the germ cells on the body wall are seen projecting into the lumen. These may be set free later, because many single germ cells were found in the lumen. In a young sporocyst, 12 days after infection, the germinal materials consist of germinal masses in different stages of development, ranging from single cells to masses containing many cells. In a sporocyst from a snail 15 days after infection, some of the embryos are much larger and become elongated, and the cellular components are no longer visible.

The mature sporocysts (Fig. 17) are also active, showing contraction and extension. The germ cells in the protoplasmic layer are not so numerous as in the younger forms. The lumen appears to be more empty. In addition to a small number of germ cells and germ balls, there are cercariae both motile and non-motile. The measurement of 15 mature sporocysts, 35 days after infection and containing motile cercariae, varies from 0.07 to 0.15 mm. by 0.98 to 2.99 mm. The width varies with the number of cercariae accumulated, as the wall is very elastic. The number of motile cercariae with eye spots was usually from two to five, but in one case 10 motile cercariae were seen.

The daughter sporocysts develop in the liver of the snail. They are usually numerous and closely packed. They are also closely associated with the host tissue, thus making it difficult to separate them. It takes 28 to 35 days for the cercariae to emerge after the snails were exposed to the miracidia.

Therefore, it takes about three weeks for the daughter sporocysts to become mature and produce the cercariae.

Cercaria: The cercariae (Fig. 18) are apharyngeal and ferocercous with pigmented eye spots. Since the morphology of the cercariae is very similar in different related bird schistosomes, many workers consider the measurement and proportions of importance. This species was measured in both ventral and lateral views.(see Table 1).

The measurement of the lateral view (taken along the dotted lines indicated in the figure (Fig.20) shows less variation. This is because the cercariae attach themselves to a surface with the ventral suckers and with the anterior and posterior ends bent away, and many of them lie on their sides after fixing, in a relaxed condition which gives less variation in measurement.

The entire surface is provided with spines. Those on the body are very fine and those on the tail are coarser and longer. The head organ is a muscular one, particularly the posterior end. The ventral sucker is subspherical. It is a very muscular and telescope-like structure, projecting and retreating. Like the body surface, it also is provided with spines.

There are five pairs of penetration glands. Two pairs of circum-acetabular glands are more glandular. The three post-acetabular glands are more homogeneous. The ducts of these glands pass forward dorsally and bend ventrally near the head organ and lying ventral to it. They open to the exterior in two groups of papillae, five in each group. Each papilla is provided with a sharp spine.

The digestive system consists of a slender, narrow oesophagus which divides a short distance posterior to the eye spots into two small sac-like caeca.

The excretory system consists of seven pairs of flame cells: six pairs in the body and one pair in the base of the tail, $2((3)+(3+1))$. There are one pair of anterior and one pair of posterior collecting tubes which are connected with each flame cell by a fine capillary tubule. The two collecting tubes on the same side of the body unite at a short distance behind and lateral to the ventral sucker into a common collecting tube which immediately gives rise to an enlarged loop containing two ciliary patches (Fig. 19 ). Each common collecting tube passes backward and inward, lying ventral to the penetration glands. Each opens into a small bladder, lying at the posterior extremity of the body. In turn, each bladder discharges into a small tube which passes into the base of the tail, around the 'island of Cort' and unites with the corresponding tube from the other side into a single common excretory tube. This common excretory tube passes through the stem of the tail and bifurcates near the posterior end of the stem. Each branch passes into a furca and eventually discharges through the tip of the furca. The anterior loop of the common collecting tube usually reaches the equator of the ventral sucker; sometimes it also reaches the anterior border or the posterior border of the ventral sucker depending on the contraction of the body. The two small excretory bladders are close together. There is a partition between the two which can easily be seen in a specimen under a cover glass and with slight pressure. This condition differs from the single bladder described by other authors.

The furcae are provided with dorso-ventral fin folds which extend from near the base of the furcae to the tips of the furcae. The width of the furcae measured from the lateral view represents their actual width. Each furca contains from 15 to 22 nuclei.

Behaviour: The cercariae are positive phototropic. They are usually found attaching to the wall of the container with their ventral suckers near the water surface on the side facing the light. If the container is disturbed and turned round, they swim actively across the container toward the lighted side and again attach. Occasionally they are seen suspended in the water with their ventral suckers attaching to the surface film. The tail and the anterior end bend away in the usual manner. Sometimes, especially after they have been in the water for a few days, they are found on the bottom of the container. In a shallow dish and under the binocular microscope they are seen attached by the powerful ventral suckers. The anterior end and the post-acetabular region together with the tail, bend backward, parallel to each other and perpendicular to the surface of attachment (Fig. 21). The furcae relax making various angles, less than 45 degrees, approaching or crossing each other. In this respect, this species is similar to that of some of the Ocellata group in Europe (Ssinitzin, 1910; Szidat, 1934). Under the cover glass the body and the tail rotate with the ventral sucker as a fulcrum. They may move on the slide with the head organ and the ventral sucker attaching and releasing alternately together with the contraction and extension of the pre-acetabular portion of the body.

While handling the cercariae the writer received the impression that the cercariae emerge after midnight or early in the morning, because if the water in the container is changed during the late evening, numerous cercariae are found the next morning. Unfortunately, when tests were to be made, the number of the cercariae produced was greatly reduced. The results gave a variable picture. In one test, the emerging of cercariae from seven experimentally infected snails was followed for 48 hours. The greatest number of cercariae produced was early in the morning and late evening. The result from another 48 hours' test was different. A large number of cercariae was produced from ten a.m. to three p.m. No peaks were reached during that evening and early the next morning. A comparatively larger number of cercariae was produced early in the morning of the third day. It seems that there is no definite time for the cercaria to emerge. Further study seems to be necessary for a more conclusive statement. However, the longevity of the cercariae discussed below may justify this irregularity.

Longevity: Several series of tests for the longevity of the cercariae were made. Infected snails were put in the beakers with unchlorinated water from the Institute well for three hours. Then the snails were removed and the beakers were subjected to different temperatures: room temperature (about 25°C.), 16 to 18°C., and about 5°C. Most of the cercariae died after three days at room temperature. At a temperature of 16 to 18°C., the cercariae lived much longer, the majority living up to four days, remaining active, and attaching to the side of the container. A small percentage lived up to five or six days, a few even longer; however, all were sluggish by then. The cercariae lived longest at a temperature of about 5°C., but due to the cold most of them were found on the bottom of the container and moving rather sluggishly. Many of them lived up to four or five days but a small percentage remained alive for from 12 days to two weeks.

## DISCUSSION

This species is very similar to both Trichobilharzia physellae as described by McMullen and Beaver (1945) and to Pseudobilharziella querquedullae as described by McLeod (1937 and 1942). McMullen and Beaver regard these two species as identical but there is a number of minor differences between them, which makes it seem desirable to retain them as separate species pending the examination of further material. There seems little doubt, however, that they are congeneric and McLeod's species is accordingly, placed in the genus Trichobilharzia as T. querquedellae (McLeod). The differences between the material under study and the two previously described forms are sufficient to describe it as new with the name Trichobilharzia cameroni.

Adults: The males and females of T. cameroni are about the same size, although the males are slightly longer, whereas there is a considerable difference in size between males and females in both T. physellae and T. querquedullae. In both of these the males are almost double the size of the females. T. cameroni has a much longer ovary in the female, a longer gynecophoral fold and a greater distance between the acetabulum and gynecophoral fold in the males than in these species. Another important character is the location of the caecal union. In T. cameroni, the caeca unite at the level of the prostate gland, similar to T. querquedullae, whereas the caecal union in T. physellae is near the anterior end of the seminal vesicle. The adults described by McLeod are much shorter than T. cameroni having larger suckers, a longer gynecophoral fold and a much greater number of testes.

Eggs: The eggs for the different species of schistosomes described so far are different. For this reason, McMullen and Beaver (1945) identified their adult parasites from Cercaria elvae as T. ocellata described by Brumpt (1931). The writer believes that eggs from different species may have a similarity in gross appearance but differ in some minor characters which are easily overlooked. The size and appearance of the egg of T. cameroni are apparently very similar to those of T. physellae and T. querquedullae. But a careful study of the drawings of the eggs made by McMullen and Beaver and by McLeod reveals some characteristic features. The eggs of T. cameroni before they absorb water have gradually tapering poles (Figs. 10a,11&12). The diagram of the egg of *T. physellae* apparently is also in this condition, since the miracidium is quite in contact with the egg shell. However, it has a very long blunt pole with the edges almost parallel and a very pointed, conical opposite pole; both poles are proportionately much longer. The egg as described by McLeod is apparently similar to that of *T. cameroni* (Fig. 10c), after water has been absorbed to some extent. It possesses two rather long poles. On the other hand, the eggs of *T. cameroni* have a constant appearance before or after the absorption of water, and the more pointed conical pole always shows its characteristic shape, except in a small number of eggs which show other variations and are abnormal.

*Cercariae*: The cercaria of *T. cameroni* has a much longer body than *Cercaria physellae*. Both species have the same length of tail stem, but *T. cameroni* has longer tail furcae, these being about two-thirds the length of the tail stem, whereas in *C. physellae* they are about one-third of the length of the tail stem. The head organ of *T. cameroni* is more slender. The ventral sucker is oval instead of round. The excretory bladder is separated, by a partition, into two, which differs from that described by Talbot (1936) for C. physellae. Above all, one of the most important characters which has been emphasized by many authors is the difference in cercarial activity. According to Cort and Talbot (1936) Cercariae physellae

> "... have a tendency to sink to the bottom of the container where they become attached. Occasionally they attach themselves to the side of the bottle but always near the bottom, and in relation to the source of light. In fact there is nothing in this activity that suggests any reaction to light either positive or negative. When at rest they assume a characteristic position with the ventral sucker attached and anterior part of the body in contact with the surface."

T. cameroni, like some of the species of the Ocellata group, attach to the surface with the ventral sucker and the pre- and post-acetabular regions bent away. They also differ from C. physellae in being positively phototropic, attaching themselves to the lighted side near the surface of the container.

The lengths of the body, tail stem, and the tail furcae, are closer to those of C. elvae Miller, 1923 (redescribed by Talbot, 1936). But the attachment of these two species is different; according to Cort and Talbot (1936),

> "... C. elvae attach themselves to the surface of the glass by the anterior tip and the protruded ventral sucker with the body between

> arched away from the furcae. The tail stem is turned back over the body and forms an acute angle ... "

The characteristic attachment of the cercariae to the container resembles three species reported by Szidat: C. neocellata and C. parocellata (1942) and C. pseudocellata (1934). C. neocellata appears to be a smaller cercaria. Its snail host is Limnaea palustris. Moreover, Szidat infected ducks with C. neocellata and recovered eggs from one of the duck's faeces like those described by Brumpt (1931) for his parasite, T. ocellata.

Cercaria pseudocellata is a slightly larger species and with a much longer tail stem and furcae. Its snail host is Limnaea palustris.

T. cameroni resembles C. parocellata in body length, but is more slender, its head organ is longer and more slender, the distance from the ventral sucker to the posterior end is much shorter, and the tail stem is also slightly shorter.

For these reasons, this species is regarded as new and the name T. cameroni, as mentioned above, was assigned to it.

For purposes of comparison, part of Szidat's Table 1 (1942) for the measurement of Cercaria neocellata, C. parocellata and C. pseudocellata; and that for Cercaria *physellae* are reproduced in Table 2. The measurements for the adults of *Trichobilharzia cameroni*, *T. physellae*, and *T. querquedullae* are shown in Table 3.

Much work has still to be carried out on the epidemiology of this parasite. From the knowledge obtained from our study, the writer has the impression that the over-wintering snails become infected with miracidia from the eggs deposited with the faeces when the migratory birds frequent the river early in the spring. Since domestic ducks even up to four months of age, become infected and retain the infection up to four months, they may play an important role in maintaining the life cycle. The cercariae, at a temperature of 16 to 18°C., remain alive up to four or five days. That might mean that the cercariae in the river can remain alive for a much longer period, as the water in the river during the summer is also close to that temperature. The infected snail was found to continue giving off cercariae for five months, when it died. The snails in the river, a natural environment, may give off cercariae for a much longer period.

## SUMMARY

The life history of *Trichobilharzia cameroni* was completed experimentally.

The eggs are spindle-shaped. The sporocysts are colourless and tubular. Mother sporocysts become mature in about a week. The younger daughter sporocyst is provided with spines on the anterior end and becomes mature in about three weeks. The period for the development of different stages in the snail varies from 28 to 35 days. The adults become mature and pass eggs, in canaries, in about 12 to 14 days.

*Physa gyrina* is the species of snail naturally infected. It was found in one case giving off cercariae for five months after being kept in the laboratory.

Domestic ducks were found to become infected until they were at least four months old, with the parasites developing to maturity in due course; no experiments were made with older ducks. Furthermore, miracidia were still recovered from the faeces four months after the duck had been experimentally infected.

BRACKETT, S.

1942 Five new species of avian schistosomes from Wisconsin and Michigan with the life cycle of _Gigantobilharzia gyrauli_ (Brackett, 1940). J. Parasitology, 28 : 25-42.

BRUMPT, E.

1931 Cercaria ocellata, déterminant la dermatite des nageurs, provient d'une bilharzie des canards. Compt. Rend. Acad. Sc., Paris, 193 : 612-614.

CORT, W. W.

1950 Studies on schistosome dermatitis. XI. Status of knowledge after more than twenty years. Amer. J. Hyg., 52 : 251-307.

CORT, W. W., AMEEL, D. J., and OLIVIER, L.

1944 An experimental study of the development of _Schistosomatium douthitti_ (Cort, 1914) in its intermediate host. J. Parasitology, 30 : 1-17.

CORT, W. W. and OLIVIER, L.

1943 The development of the sporocysts of a schistosome, _Cercaria stagnicolae_ Talbot, 1936. J. Parasitology, 29 : 164-176.

CORT, W. W. and TALBOT, S. B.

1936 Studies on schistosome dermatitis. III. Observations on the behavior of the dermatitis-producing schistosome cercariae. Amer. J. Hyg. 23 : 385-396.

EJSMONT, L.

1929 Ueber zwei Schistosomatidengattungen der Vögel. Bull. Intern. Acad. Polon. Sc. et Lett., Cracovie, Cl. Sc. Math. et Nat. s. B. : Sc. Nat. (II) : 389-403.

FAUST, E. C. and MELENEY, H. E.

1924 Studies on Schistosomiasis japonica. Amer. J. Hygiene, monographic series No. 3, 339 pp.

McLEOD, J. A.

1937 Two new schistosomid trematodes from water-birds. J. Parasitology, 23 : 456-466.

1940 Studies on cercarial dermatitis and the trematode family Schistosomatidae in Manitoba. Can. J. Research, D,18 : 1-28.

McLEOD, J. A. and LITTLE, G. E.

1942 Continued studies on cercarial dermatitis and the trematode family Schistosomatidae in Manitoba. Can. J. Research, D,20 : 170-181.

McMULLEN, D. B. and BEAVER, P. C.

1945 Studies on schistosome dermatitis. IX. The life cycles of three dermatitis-producing schistosomes from birds and a discussion of the subfamily Bilharziellinae (Trematoda : Schistosomatidae). Amer. J. Hygiene, 42 : 128-154.

MILLER, Jr., H. M.

1923 Notes on some furcocercous larval trematodes. J. Parasitology, 10 : 35-46.

OISO, T.

1927 On a new species of avian schistosoma developing in the portal vein of the duck, and an investigation of its life-history. Taiwan Igakkai Zasshi (270) 848-865 (English summary pp. 1-3).

PRICE, E. W.

1929 A synopsis of the trematode family Schistosomatidae with descriptions of new genera and species. Proc. U. S. Nat. Museum, 75, Art. 18, 39 pp.

PRICE, H. F.

1931 Life history of Schistosomatium douthitti (Cort). Amer. J. Hygiene, 13 : 685-727.

SZIDAT, L.

1938 Pseudobilharziella filiformis n. sp. eine neue Vogelbilharzie aus dem H"ockerschwan Cygnus olor L. Zeit. f. Parasitenk., 10 : 535-548.

1942 Was ist Cercaria ocellata La Vallette? Morphologische und entwicklungsgeschichtliche untersuchungen über den Erreger der Europäischen Cercarien-Dermatitis des menschen. Deutsch. Trop. Zeit., 46 : 481-497, 504-524.

SZIDAT, L. and WIGAND, R.

1934 Leitfaden der einheimischen Wurmkrankheiten des Menschen. Leipzig, 212 pp. (Schistosome dermatitis, pp. 72-76).

TALBOT, S. B.

1936 Studies on schistosome dermatitis. II. Morphological and life history studies on three dermatitis-producing schistosome cercariae, C. elvae Miller, C. stagnicolae n. sp., and C. physellae n. sp. Amer. J. Hygiene, 23 : 372-384.

YAMAGUTI, S.

1941 Studies on the helminth fauna of Japan. Part 32. Trematodes of birds. V. Jap. J. Zoology, 9 : 321-341.

TABLE 1. MEASUREMENT OF THIRTY CERCARIAE

| | Ventral View | | | | Lateral View | | | |
|---|---|---|---|---|---|---|---|---|
| | Maximum | Minimum | Mean | Standard Deviation | Maximum | Minimum | Mean | Standard Deviation |
| | mm. | mm. | mm. | mm. | mm. | mm. | mm. | mm. |
| Length of body | 0.361 | 0.257 | 0.319 | ±0.0256 | 0.347 | 0.296 | 0.329 | ±0.0131 |
| Width of body | 0.070 | 0.052 | 0.057 | ±0.0040 | 0.059 | 0.049 | 0.054 | ±0.0026 |
| Length of head organ | 0.132 | 0.080 | 0.108 | ±0.0113 | 0.125 | 0.090 | 0.110 | ±0.0119 |
| Width of head organ | 0.046 | 0.028 | 0.038 | ±0.0038 | 0.044 | 0.035 | 0.038 | ±0.0018 |
| Length of ventral sucker | 0.031 | 0.024 | 0.030 | ±0.0020 | 0.035 | 0.031 | 0.032 | ±0.0011 |
| Width of ventral sucker | 0.035 | 0.028 | 0.032 | ±0.0024 | | | | |
| Distance from anterior end to ventral sucker | 0.219 | 0.139 | 0.187 | ±0.0215 | 0.237 | 0.184 | 0.209 | ± 0.0111 |
| Distance from posterior end to ventral sucker | 0.111 | 0.073 | 0.090 | ±0.0119 | 0.104 | 0.077 | 0.086 | ± 0.0062 |
| Length of tail stem | 0.424 | 0.347 | 0.369 | ±0.0242 | 0.382 | 0.344 | 0.363 | ±0.0106 |
| Width of tail stem | 0.042 | 0.035 | 0.038 | ±0.0015 | 0.040 | 0.031 | 0.037 | ± 0.0022 |
| Length of tail furca | 0.253 | 0.195 | 0.225 | ±0.0114 | 0.240 | 0.195 | 0.220 | ± 0.0092 |
| Width of tail furca | 0.026 | 0.019 | 0.023 | ±0.0014 | 0.038 | 0.029 | 0.033 | ±0.0070 |

TABLE 2 MEASUREMENTS OF CERCARIAE

| | *Cercaria neocellatta* | C. *parocellata* | C. *pseudocellatta* | C. *physellae* |
|---|---|---|---|---|
| | mm. | mm. | mm. | mm. |
| Length of body | 0.27 | 0.34 | 0.37 | 0.265 ± 0.0084 |
| Width of body | 0.055 | 0.07 | 0.08 | 0.060 ± 0.0046 |
| Head organ length/width | 0.085/0.04 | 0.09/0.05 | 0.11/0.06 | 0.095 ± 0.0050 by 0.039 ± 0.0020 |
| Ventral sucker length/width | 0.026 | 0.035 | 0.045 | 0.029 ± 0.0024 |
| Distance of ventral sucker to posterior end of body | 0.1 | 0.125 | 0.17 | 0.080 ± 0.0052 |
| Tail stem length/width | 0.36-0.39/ 0.036 | 0.4-0.44/ 0.04 | 0.58/0.05 | 0.374 ± 0.0106 by 0.040 ± 0.0036 |
| Tail furca length/width | 0.23/0.02 | 0.24/0.025 | 0.31/0.028 | 0.196 ± 0.0078 by 0.032 ± 0.0009 |

TABLE 3. MEASUREMENTS OF MALES

| | Trichobilharzia cameroni | T. physellae | Pseudobilharziella querquedullae |
|---|---|---|---|
| | mm. | mm. | mm. |
| Body length | 3.18-5.71 | Up to 7.5 | 3.7 |
| Body width | 0.063-0.097 | | 0.150 |
| Oral sucker | 31-40 x 38-47µ | 24-28 x 28-40µ | 56 x 64µ |
| Ventral sucker | 31-49 x 49-51µ | 16-32µ diam. | 73µ (immature) |
| Distance between oral and ventral suckers | 0.226-0.370 | 0.16-0.34 | 0.274 from anterior end |
| Distance between ventral sucker and gynecophoral fold. | 0.230-0.536 | 0.14-0.34 | 678u from anterior end |
| Testes - number | about 70-110 | 96-160 | 210-240 |
| Length of gynecophoral fold | 0.212-0.345 | 0.10-0.19 x 0.056-0.080 | 0.375 (immature) |
| Intestinal reunion | Level of prostate gland | Near anterior end of seminal vesicle (about equal distance as intestinal bifurcation from acetabulum | in region of gynecophoral fold just caudad of genital pore |

TABLE 3 (cont'd) MEASUREMENTS OF FEMALE

| | *Trichobilharzia cameroni* | *T. physellae* (McMullen & Beaver) | *Pseudobilharziella querquedulla* (McLeod) |
|---|---|---|---|
| | mm. | mm. | mm. |
| Body | | | |
| Length | 3.82-4.94 | Up to 4.4 | 1.86 |
| Width | 0.052-0.059 | 0.040-0.080 | 60µ |
| Oral sucker | 0.025-0.034 x 0.032-0.040 | 0.024 x 0.024 to 0.044 x 0.028 | 0.034 |
| Acetabulum | 0.025-0.038 long x 0.030-0.047 wide | 0.024-0.032 to 0.040 protruded | 0.036 |
| Distance between anterior end and acetabulum | 0.213-0.342 | 0.2-0.3 | 0.250 |
| Distance between acetabulum and ovary | 0.179-0.250 | 0.1-0.3 | 0.170 |
| Ovary | | | |
| Length | 0.287-0.358 | 0.09-0.20 | 0.120 (overall) |
| Width | 0.016-0.033 | 0.020-0.072 | 0.056 |
| Seminal Receptacle | | | |
| Length | 0.072-0.126 | | |
| Width | 0.018-0.042 | | |
| Eggs | | | |
| Saline | 57 x 147µ to 73 x 181µ 63 x 212µ | 0.170 x 0.065 to 0.250 x 0.080 | 0.217 x 0.076 |
| Water | 66 x 198µ to 77 x 253µ 80 x 240µ | | |
| Cecal reunion | at level of seminal receptacle | posterior to seminal receptacle. | |
| Papilla of Laurer canal | Just in front of seminal receptacle | Just back of ovary | Just posterior to ovary |

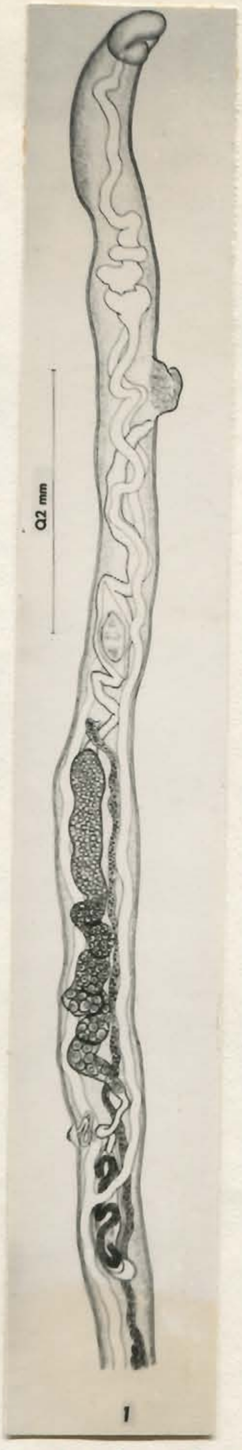



Fig. 1. Anterior end of a female <u>Trichobilharzia cameroni</u> sp. nov.


Fig. 2. Anterior end of a male Trichobilharzia cameroni sp. nov.

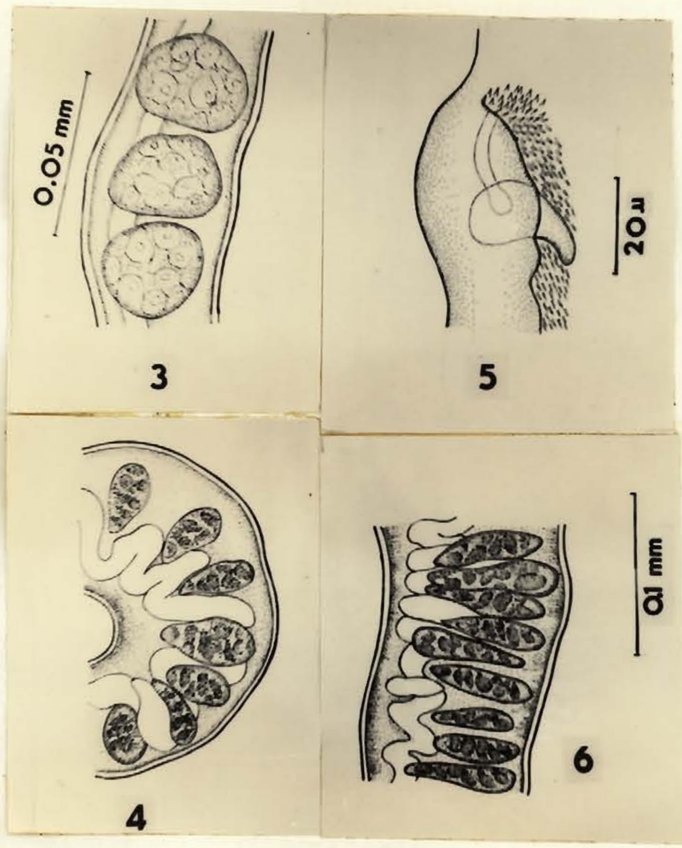
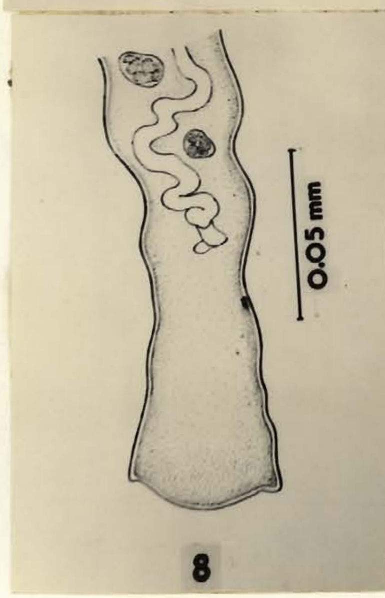



Fig. 3. A portion of the posterior end of an extended male, showing the normal shapes and the linear arrangement of the testes.

Fig. 4. A portion of a male, bending, showing the intestinal caecum and the club-shaped testes due to pressure.

Fig. 5. The anterior right portion of the gynecophoral fold showing the spines and the genital papilla.

Fig. 6. A contracted portion of a male, showing the caecum and the elongated testes due to contraction.

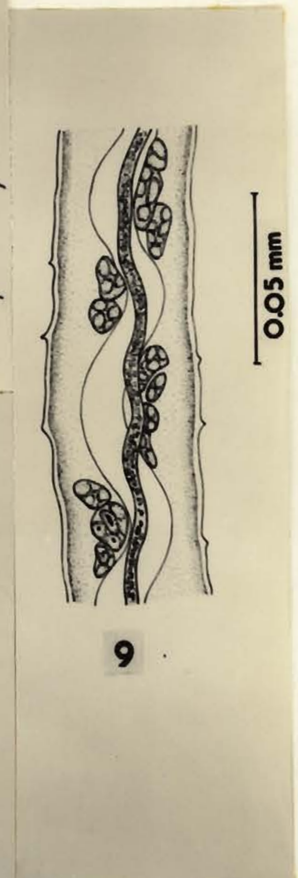
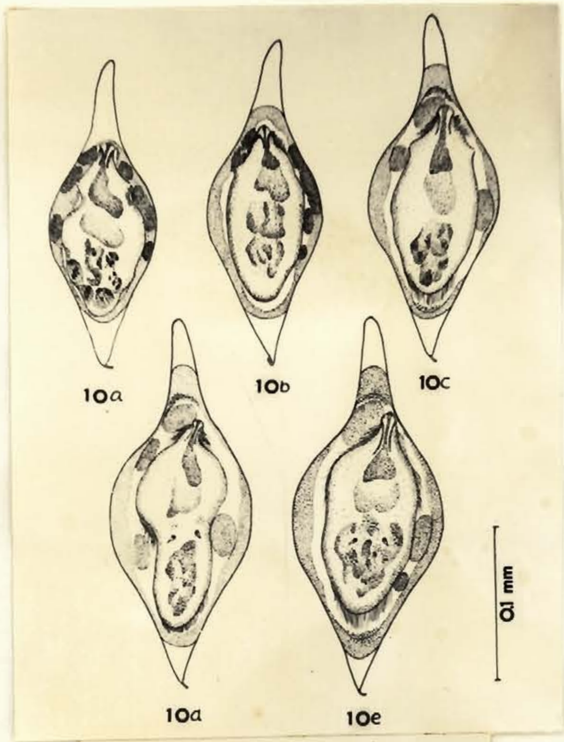




Fig. 7. A contracted gynecophoral fold of a male,
Fig. 8. Posterior end of male showing two testes and the caecum.
Fig. 9. A portion of a female showing the vitelline duct and groups of vitellaria alternating with the caecum.



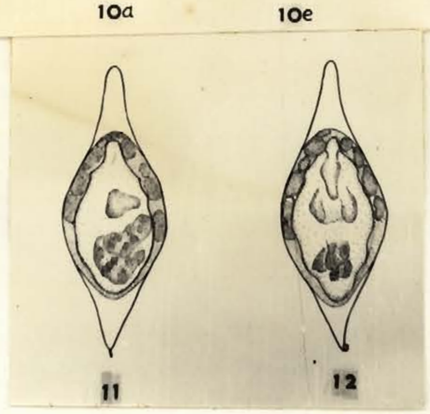




Fig. 10a. A normal egg in saline.
10b. The same egg, 20 minutes after saline was replaced with water.
10c. 30 minutes in water.
10d. 37 minutes in water.
10e. 43 minutes in water.
Fig. 11 and 12. Two normal eggs in saline.

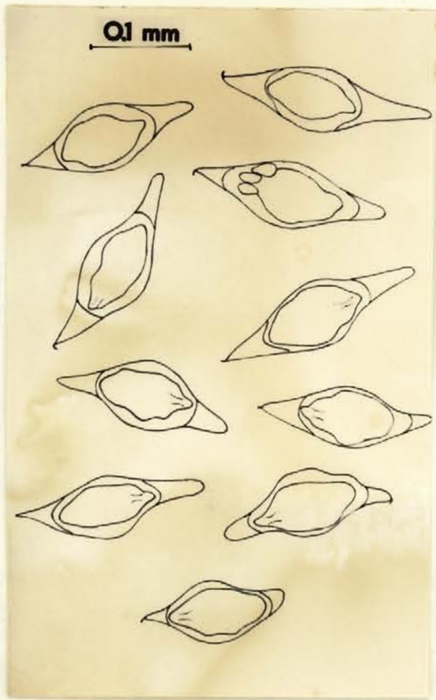



Fig. 13. Ten random abnormal eggs showing variations in shape.

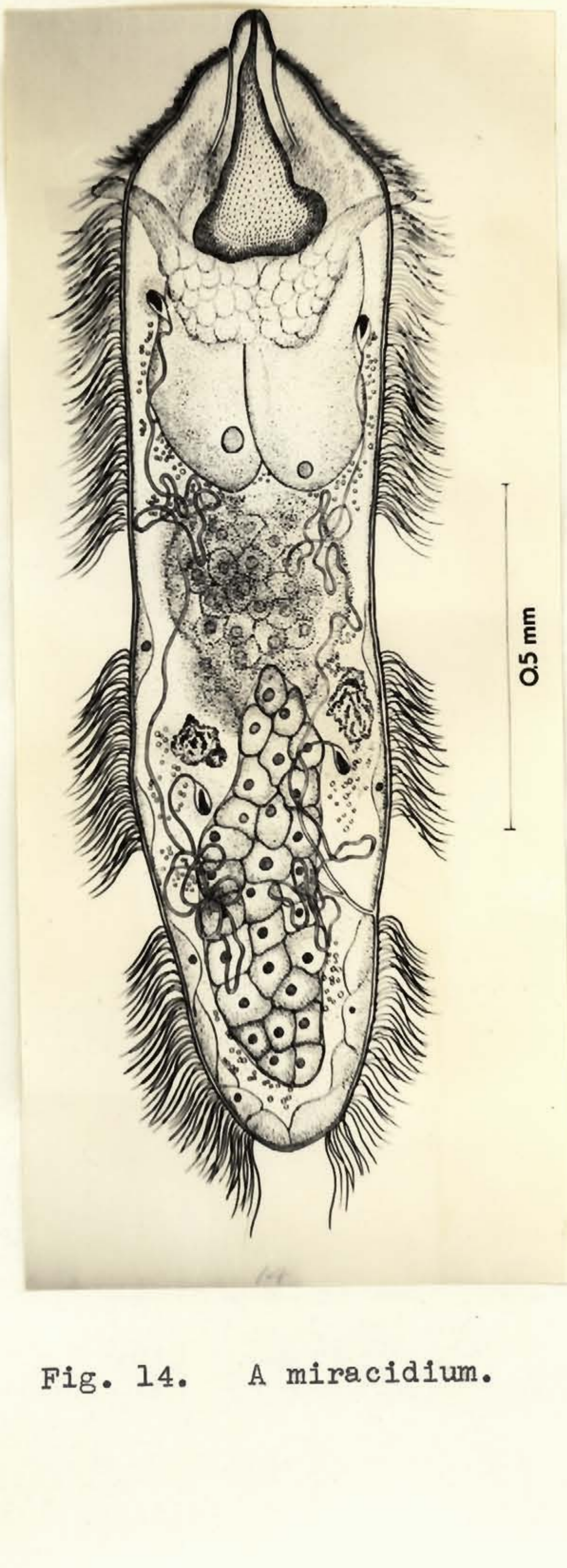



Fig. 14. A miracidium.

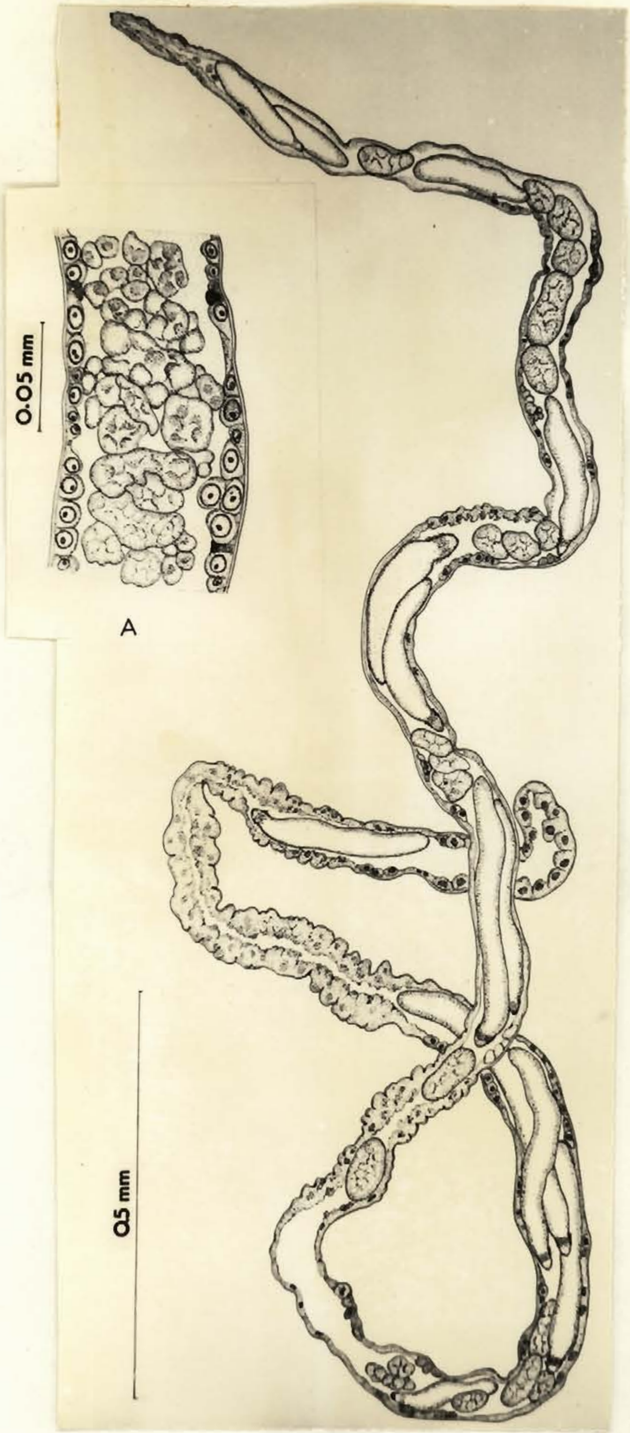



Fig. 15a. A portion of a mother sporocyst, 9 days after infection, showing the germ balls and germ cells embedded in the wall of the sporocyst.

Fig. 15. A mature sporocyst, from an experimentally infected snail with migrating second daughter sporocyst and germ balls.

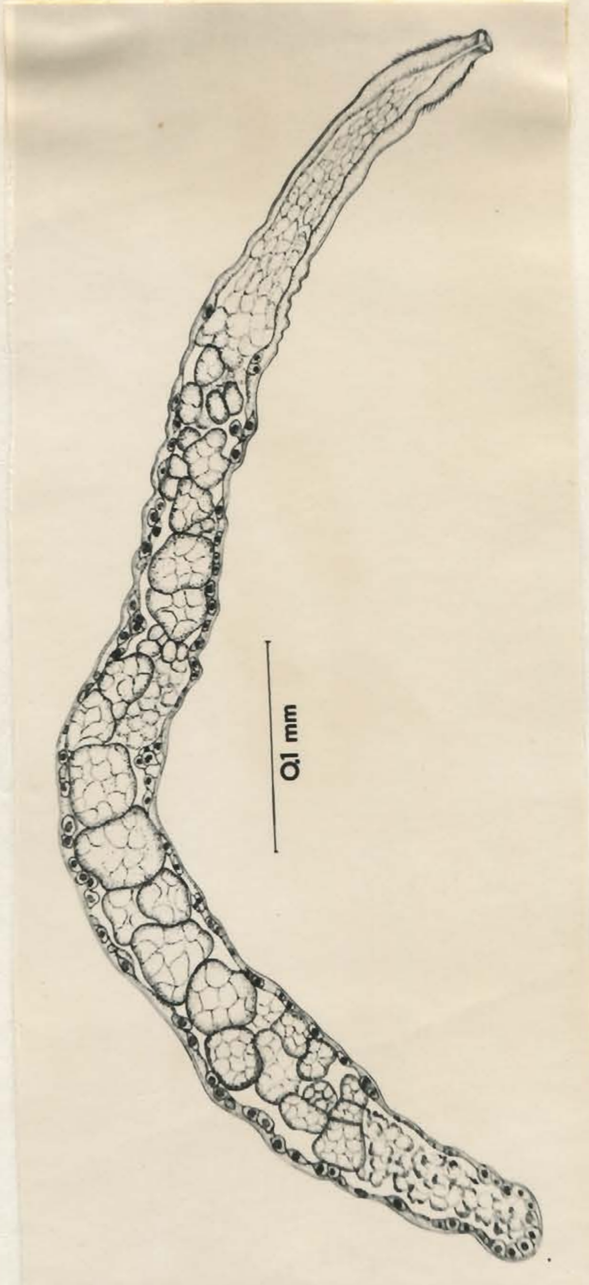



Fig. 16. A young daughter sporocyst from an experimentally infected snail, showing germ cells, germ masses and spines at the anterior end.

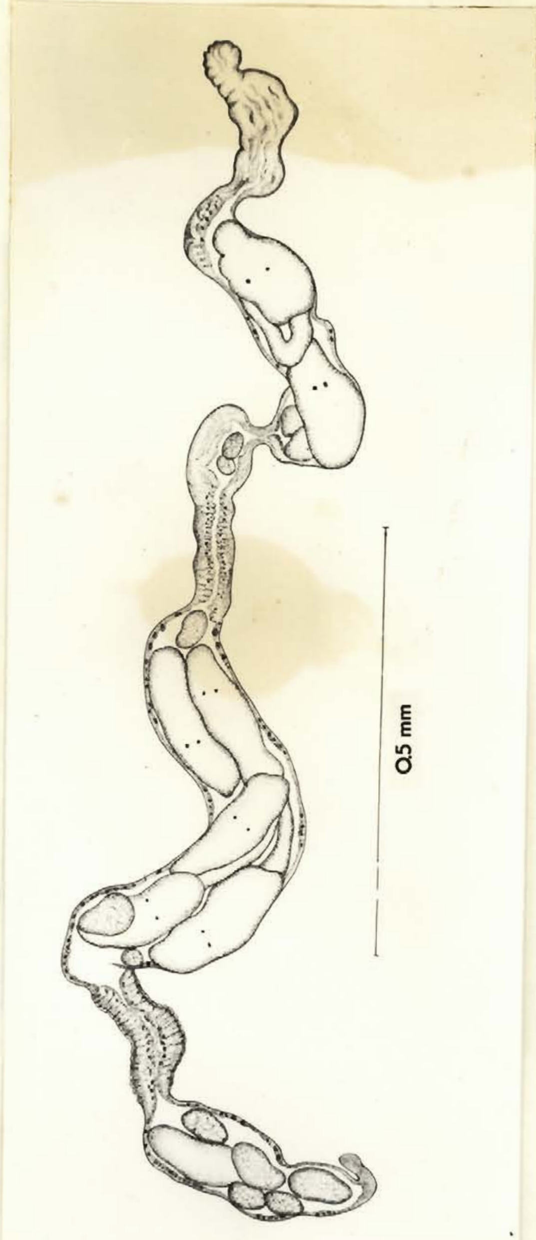



Fig. 17. A mature daughter sporocyst from an experimentally infected snail, showing germ masses and the migrating cercaria.

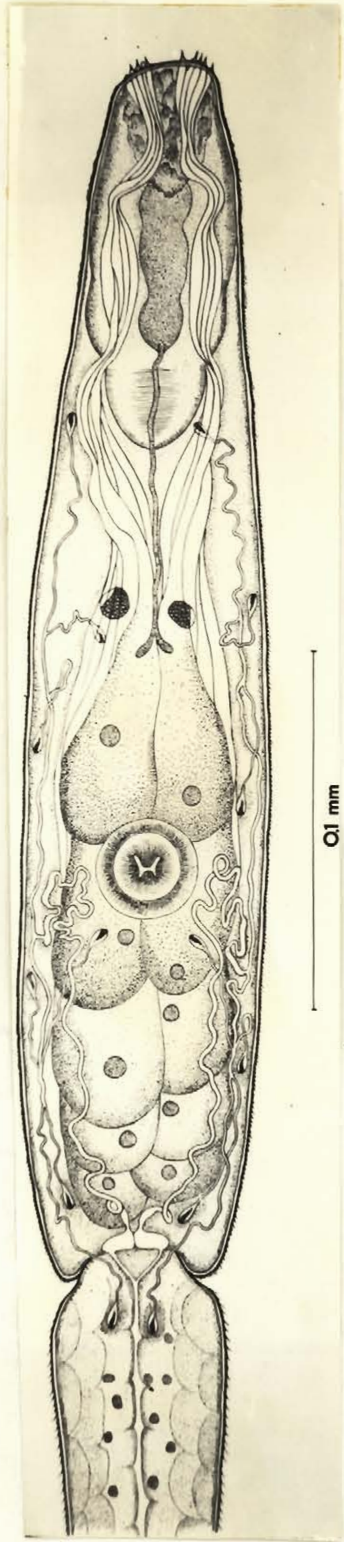



Fig. 18. The body of a cercaria with the basal portion of its tail.

Fig. 19. An enlarged sketch of the cilliary patches.

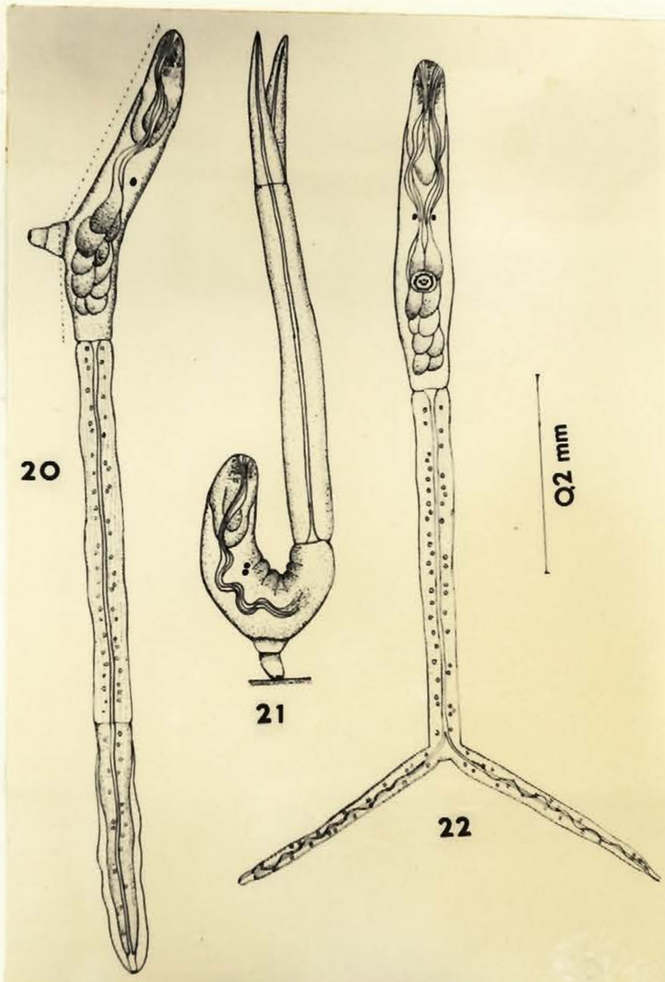



Fig. 20. Lateral view of a formaline preserved cercaria.
Fig. 21. A living cercaria attaching to the substratum with its ventral sucker and with the pre-acetabular region and tail bending away.
Fig. 22. Ventral view of a fairly well extended formaline preserved cercaria.

## PART II. A STUDY OF THE LIFE HISTORIES OF FOUR NEW SPECIES OF PLAGIORCHIS (FAMILY PLAGIORCHIDAE)

CONTENTS

# INTRODUCTION

During the summers of 1950 and 1951, Stagnicola palustris, one of the most common snails in the Ottawa River near Ste. Anne de Bellevue, near Montreal, was collected for cercarial study. This species of snail was found commonly infected with Xiphidiocercariae of the Polyadena group (Sewell, 1922). Both a morphological study and experimental infections were made. Adults recovered all belonged to the genus Plagiorchis.

From this study, the writer has an impression that this genus will eventually contain a tremendous number of species; it is already a very large genus. Even in this small area, at least six morphologically different cercariae were recovered from snails and adults of four of these were recovered from pigeons. Apparently their natural hosts are birds. Another species was recovered from the caecum of a white mouse; the specimens were mature but very small and the white mouse may not be a suitable host. The specimens were not well preserved so a detailed study could not be made. The sixth species failed to develop in pigeons.

From this life history study, several characters in the cercarial and metacercarial stages appear to have systematic importance. The sizes of the cercariae of different species are quite different in formalin-fixed material. But, due to contraction, they are subject to great variations. However, the differences are difficult to observe in the living specimens. On the other hand, the appearance of the cercariae due to the content of body is characteristic. It may appear clear, finely granulated, coarsely granulated and darker, or have various amounts of large, clear globules, as oil droplets, of various sizes. The appearance of the body is specially valuable for one who has some experience with the cercariae. The size of the penetration stylet will only serve for identification to a certain extent, as some species may have the same size of stylet. The depressed area at the caudal end, where the tail is attached, may be smooth or have tooth-like structures giving a comb-like appearance. The activity of the cercariae on the slide is also characteristic. Some cercariae tend to swim when there is plenty of water. Others tend to attach to and move on the slide with their suckers. The combination of these characters is valuable for differentiation purposes.

The size of the cyst is very constant for each species, at least for the four species under study. The shape of the excretory bladder and the colour are also, to a certain extent, characteristic.

*Plagiorchis* is a large genus, containing about 75 species. The extensive systematic study of this genus by Olsen (1937) provided a valuable aid for future studies of this large group. Twenty-two more species have been reported since 1937. The descriptions of five of them, *Plagiorchis magnacotylus* and *P. oriental* (from Korea, Park, 1939), *P. morosovi* and *P. ptschelkini* (from Russia, Sobolev, 1946), and *P. microti* (from Russia, Soltys, 1949) are not available to the writer.

## MATERIAL AND METHOD OF STUDY

Snails were collected from the Ottawa River. These were identified by Professor John Oughton of the Ontario Agricultural College, Guelph, Ontario, as *Stagnicola palustris*. Naturally infected snails were separated in different containers. Cercariae emerging from such snails were examined. Those appearing to be different, based on the characters mentioned above, were selected for experimental study.

The larvae of dragon flies, damsel flies, and water beetles were collected from ponds where no Xiphidiocercariae were found. All these insect larvae served as intermediate hosts, although dragon fly larvae were chiefly used.

Cercariae were stained with very dilute neutral red, and fixed with hot formalin, for measurement. Their morphology was studied with living material. The metacercariae were studied and measured in a living condition.

## PLAGIORCHIS STAGNICOLAE SP. NOV.

### Adult

The body is elongated and slightly flattened dorso-ventrally. The margins of the body are almost parallel (Fig. 1). The cuticle is covered with fine spines at the anterior end. Measurements of 18 specimens, nine days old, from an experimentally infected pigeon, vary from 1.17 to 1.832 mm. in length and 0.285 to 0.375 mm. in width. The oral sucker is subterminal and subspherical; it is 0.146 to 0.171 mm. in width and 0.146 to 0.184 mm. in length. The ventral sucker is located at the anterior third of the body. It is much smaller than the oral sucker, slightly wider than long, varying from 0.108 mm. to 0.136 mm. in width and 0.104 mm. to 0.125 mm. in length. A prepharynx is present, but is very short. The pharynx is muscular, wider than long, and four-lobed anteriorly. It measures 0.066 to 0.084 mm. in length and 0.084 to 0.101 mm. in width. The oesophagus is not seen. The intestinal caeca are broad and end a short distance from the posterior end of the body.

The genital pore is in front of the ventral sucker and slightly to the left. The ovary is about the size of the ventral sucker, subspherical to irregularly oval. It is usually located a short distance behind the ventral sucker and slightly to the right of the midline, although in some specimens its border almost touches the ventral sucker. It measures from 0.104 to 0.139 mm. in width and from 0.125 to 0.153 mm. in length. The shell gland mass is immediately behind the ovary and on the mid-line. The uterus is fairly well developed, extending to the post-testicular region. It turns forward at different levels mid-way behind the posterior testis and the posterior end of the body. In most of the specimens the loops reach the posterior extremity behind the ends of the caeca. A matraterm is present and on the left side. The vitellaria are very well developed with very large follicles, usually extending from the level between the posterior border of the pharynx and the intestinal bifurcation to the posterior extremity of the body and beyond the ends of the intestinal caeca; in some specimens the follicles extend further forward to about the middle of the pharynx. The follicles occupy the lateral fields of the body, overlapping practically the whole length of the caeca. The follicles meet dorsally in the post-testicular region and occupy most of the inter-caecal space. In front of the ventral sucker, the follicles from both sides extend inward dorsally and almost meet at midline, leaving only a small gap between them; in one specimen, the follicles from each side actually met.

The eggs are yellowish brown and operculated (Fig. 17a), varying from 0.033 to 0.038 mm. in length and 0.021 to 0.023 mm. in width.

The testes are about the same size as the oral sucker and are oval-shaped. The anterior testis is immediately behind the shell gland. It measures 0.110 to 0.174 mm. wide and 0.150 to 0.191 mm. long. The posterior testis is immediately behind the anterior testis and slightly to the right side of the midline. It is larger, measuring 0.121 to 0.174 mm. in width and 0.164 to 0.223 mm.

in length. The ovary and the testes occupy about the middle third of the body. The cirrus sac is large and measures from 0.270 to 0.385 mm. in length and with a diameter of about 0.060 mm., about half the diameter of the ventral sucker. It curves slightly to the right side, dorsal to and on the right side of the ventral sucker, and with the distal portion bending to the left side of the body. The basal portion of the sac is left of the ovary or overlaps the left half of the ovary. The base of the sac is about the level of the middle of the ovary. The seminal vesicle is constricted into a spherical anterior portion and an elongated posterior portion which is contracted and appears to be oval-shaped in the mounted specimen. The cirrus is long and protrusible.

The excretory system is Y-shaped. The unbranched stem is very long, reaching the anterior border of the anterior testis where it receives two common collecting tubes. Only the basal portions of these two collecting tubes are seen, about the same diameter as the unbranched stem. They can be followed up to the sides of the ovary. The excretory pore is ventral and subterminal.

Cercaria

The cercariae were obtained from naturally infected *Stagnicola palustris*. Numerous cercariae emerged from the snail every day. The cuticle is covered with fine spines. The body is quite clear (Fig. 6). The measurements of 25 cercariae vary from 0.202 to 0.258 mm. long and 0.066 to 0.104 mm. wide. The size depends chiefly upon the state of contraction; when it is extended, it is much narrower in width.

The caudal end of the body has a round depressed area on the ventral surface where the tail is attached. This depressed area is smooth without tooth-like structures. The tail is from 0.017 to 0.021 mm. wide and 0.139 to 0.174 mm. long.

The oral sucker is subterminal and fairly large. It varies from 0.039 to 0.045 mm. in width and 0.045 to 0.050 mm. in length. The penetration stylet seen dorsal to the oral sucker, is 31 $\mu$ long and 6.0 $\mu$ wide at the base. The ventral sucker is much smaller than the oral sucker, located just behind the middle of the body. It is 0.030 to 0.035 mm. wide and 0.031 to 0.036 mm. long.

Both the prepharynx and the pharynx are

present. Intestinal caeca could not be traced. There are at least seven pairs of penetration glands with two bundles of ducts, one on each side of the oral sucker, leading to the base of the penetration stylet. The excretory bladder is Y-shaped. It consists of a subspherical basal portion with a very thick wall and two enlarged basal portions of the two common collecting tubes. The unbranched portion of the common collecting tube is absent.

Dragon fly larvae were chiefly used for cercarial penetration. They were placed together in a container, usually for a day. The cercariae crawled around the body of the insect larvae and penetrated the thinner part of the cuticle between the joints and the segments. As with other cercariae, the tail was detached when penetration was in progress. Encystment took place in both the head and the body and usually between the muscle and the subcuticle.

Metacercaria

The cysts are subspherical and clear (Fig. 10). The trilobed excretory bladder is filled with large dark granules. Measurement of 12 cysts, living and about a week old, under a cover glass, varied from 0.139 to 0.150 mm. and 0.146 to 0.153 mm. in diameter. The cuticle is covered with spines which are much larger on the anterior end and become very fine on the posterior end. The stylet is present but detached and set free inside the cyst cavity.

Experimental Definitive Host

One pigeon was experimentally infected with 130 cysts about a week old, from dragon fly larvae. The bird was killed nine days after infection. It was found to be heavily infected. About one hundred parasites were found in the posterior third of the small intestine,

One white mouse was fed with 140 cysts about a week old, and fed again with 75 cysts about five days later. It was killed ten days after the last infection. The result was negative. Another white mouse was fed with 20 cysts, 23 days old and was killed four days later. Three young flukes were recovered from the intestine in front of the caecum.

Miracidium and Sporocyst

To prevent any loss of eggs during handling, about 40 parasites obtained from the pigeon were left in water in a petri dish and the water was changed frequently. The embryos in the eggs were in the four-celled stage on the 2nd July, the day on which the bird was killed. One week later, the embryo had a large vacuole at the centre and cells were no longer seen. On the 13th July, a dead worm was pressed under a cover glass. The eggs showed miracidia moving inside. The remaining parasites were teased into small fragments in a finger bowl containing water. Experimental infection was made with laboratory-bred snails, Stagnicola palustris. Twenty-one snails were placed with the fragments of the parasites. The snails were directed so as to glide over the fragments and were allowed to feed on the bottom of the container for a few hours. On the 26th July, three snails were examined but all were negative. On the 13th August, four snails were examined. One was found infected. Some young sporocysts were found attached to the digestive tract but most of them were in or on the liver. On the 20th August, 37 days after infection, ten snails were examined and one of them was found infected. Most of the sporocysts had from two to four motile cercariae.

The bodies of these cercariae were filled with large clear globules.

The sporocysts are tubular in shape (Fig. 14). Measurements of ten fixed specimens varied from 0.125 to 0.190 mm. in maximum width and 0.808 to 1.144 mm. in length.

The life history of P. stagnicolae was completed in the laboratory. It takes about eight to ten days for the eggs to develop into miracidia and about five weeks for the sporocysts to produce cercariae. The metacercariae developed favourably in the pigeon and required about nine days to reach maturity.

## PLAGIORCHIS PALUSTRIS SP. NOV.

### Adult

The body is elongated and narrow at both ends (Fig. 2). The cuticle is covered with fine spines at the anterior end. Measurements from six specimens (11 days old) varied from 0.375 to 0.420 mm. in width and 1.660 to 2.112 mm. in length.

The oral sucker is subspherical and subterminal, varying from 0.160 to 0.195 mm. wide and 0.181 to 0.195 mm. long. The ventral sucker is smaller than the oral sucker, varying from 0.111 to 0.157 mm. wide and 0.139 to 0.153 mm. long. The pharynx is 0.087 to 0.101 mm. wide and 0.087 to 0.104 mm. long. It is four-lobed anteriorly. Neither prepharynx nor oesophagus is seen. Intestinal caeca arch forward then turn backward, ending a short distance from the posterior end.

The genital pore is in front of the ventral sucker and slightly to the left of the midline. The ovary is about equal to or slightly larger than the ventral sucker. It varies from 0.111 to 0.167 mm. wide and 0.150 to 0.177 mm. long. It is located very close to the ventral sucker and slightly to the right. The shell gland mass is immediately behind the ovary and on the midline. The uterus reaches the posterior end of the body beyond or in front of the ends of the intestinal caeca. The vitellaria are well developed with very large follicles, extending from about midway between the oral and ventral suckers to the posterior tip of the body, overlapping the intestinal caeca. The follicles meet dorsally in the post-testicular region and occupy most of the space. In front of the ventral sucker, the follicles do not meet dorsally.

The eggs are operculated and vary from 20 to 23 $\mu$ by 35 to 39 $\mu$ (Fig. 17b).

The testes and the ovary are in a curved line, equidistant from each other, and occupy the middle third of the body. The anterior testis varies from 0.101 to 0.122 mm. wide and 0.146 to 0.164 mm. long. The posterior testis is slightly larger, varying from 0.104 to 0.132 mm. wide and 0.174 to 0.191 mm. long. The cirrus sac is only slightly curved, lying dorsal to the ventral sucker with its base about the middle of the ovary. It is 0.087 to 0.110 mm. wide and 0.320 to 0.360 mm. long. The cirrus is very long and protrusible.

The excretory pore is ventral and subterminal.

<u>Cercaria</u>

When the cercariae were placed in a drop of water on a slide, they attached firmly to the slide and moved with their suckers. The body is very granulated, giving a much darker and greenish appearance than the other species studied (Fig. 7). Furthermore the entire body is filled with a large number of globules of various sizes, making observation of the penetration glands difficult. However, there are between five to eight pairs of these. Measurements of 20 cercariae varied from 0.090 to 0.115 mm. wide and 0.209 to 0.302 mm. long. The tail is 0.185 to 0.238 mm. long and about 0.024 mm. at the base. The caudal cavity where the tail attaches, is provided with teeth-like structures. The cuticle is covered with spines.

The oral sucker is subspherical varying from 0.045 to 0.053 mm. wide and 0.048 to 0.058 mm. long. The penetration stylet is large, about 38 $\mu$ long and 7 $\mu$ at the base. The ventral sucker is much smaller, varying from 0.034 to 0.038 mm. wide and 0.031 to 0.036 mm. long. Prepharynx and pharynx present.

The excretory system is Y-shaped. The bladder is very muscular with some tooth-like structures at its bottom. The unbranched common collecting tube is fairly long.

The cercariae, as was the case with the other species, encysted in dragon fly larvae.

<u>Metacercaria</u>

The cysts are oval, full of clear globules of various sizes (Fig. 11). The excretory bladder is trilobed. It is a reasonably dark colour, but is not solidly black. Measurements of 12 metacercariae, eight days old, vary from 0.153 to 0.171 mm. in length and 0.191 to 0.223 mm. in diameter.

Experimental Definitive Host

One hundred cysts, eight days old, from experimentally infected dragon fly larvae, were fed to a pigeon. Two days later, another 47 cysts, also eight days old, were again fed. Six days after the initial feeding, the faeces of the bird were examined and eggs of the parasites were recovered. The bird was killed 11 days after the first feeding. Ten adults were recovered from the posterior portion of the intestine.

## PLAGIORCHIS CANADENSIS SP. NOV.

### Adult

The body is elongated (Fig. 3). Measurement of five slightly pressed mounted specimens, from the pigeon, 11 days after infection, varied from 0.480 to 0.555 mm. in width and 1.712 to 2.312 mm. in length. The anterior end of the body is covered with coarse spines which become gradually reduced in size and disappear at the level of the posterior testis.

The oral sucker is subterminal, slightly longer than wide, measuring from 0.167 to 0.195 mm. wide and 0.174 to 0.202 mm. long. The ventral sucker is 0.153 to 0.181 mm. wide and 0.164 to 0.174 mm. long. Its anterior border is from 0.360 to 0.465 mm., about one-quarter to one-fifth the body length, from the anterior end of the body. A prepharynx is present. The pharynx is four-lobed anteriorly, slightly wider than long. It varies from 0.090 to 0.104 mm. wide and 0.087 to 0.090 mm. long. An oesophagus was not seen. The intestinal caeca arch slightly forward at the sides of the pharynx, then turn backward ending a short distance from the posterior end.

The genital pore is immediately in front of the ventral sucker and slightly left of the midline. The ovary is smooth in outline. It measures from 0.143 to 0.184 mm. in width and 0.195 to 0.244 mm. in length. It is located a short distance behind the ventral sucker and slightly to the right of the midline. The shell gland mass is immediately behind the ovary. A metraterm is present. The uterus in most of the specimens reaches the posterior end of the body, beyond the tip of the caeca, but in one it turns forward at about the middle of the posterior testis. The vitellaria are well developed with large follicles, extending from the posterior border of the pharynx to the posterior end of the body. They occupy the lateral fields of the body overlapping the intestinal caeca. The follicles extend inward and meet dorsally in the post-testicular region. In front of the ventral sucker, the follicles from both sides meet and form a band. Eggs are operculated varying from 0.038 to 0.042 mm. long and 0.021 to 0.024 mm. wide (Fig. 17c).

The testes are irregularly oval, with smooth margins, and are much larger than the ovary. They occupy the posterior half of the body. They are well separated from each other and are slightly obliquely located. The anterior testis is 0.216 to 0.247 mm. wide and 0.253 to 0.354 mm. long. The posterior testis is slightly larger and is located at from 0.285 to 0.345 mm. (about one-seventh to one-sixth the body length) from the posterior end. It is from 0.219 to 0.237 mm. wide and 0.264 to 0.395 mm. long. The cirrus sac is elongated and curved. The anterior portion lies dorsal to the ventral sucker and to the right of the midline. The posterior end bends toward the left side of the body with its base ending between the middle and the posterior margin of the ovary. In two mounted specimens, the cirrus sacs are 0.550 and 0.610 mm. long, respectively. Measurement from four fresh specimens, under cover glass, varied from 0.122 to 0.140 mm. in maximum width and 0.760 to 880 mm. in length. The maximum diameter is, therefore, slightly over half of the diameter of the ventral sucker, which is about 0.240 mm. in diameter in the fresh condition, and under pressure. The seminal vesicle is constricted into an anterior subspherical and an elongated posterior portion. The cirrus is very long. A short protrusion is seen in one specimen.

The excretory system, like other species, is Y-shaped. The unbranched collecting tube is very long. It extends forward to the level of the anterior border of the anterior testis where it receives the two lateral collecting tubes. The excretory pore is ventral and subterminal.

Cercaria

The body is clear and contains a small

number of clear globules (Fig. 8). The cuticle is covered with fine spines. The length and width of the body varies, approximately in inverse proportion to the state of contraction. It is from 0.083 mm. wide and 0.160 mm. long to 0.059 mm. wide and 0.237 mm. long.

The oral sucker is spherical to subspherical, measuring 0.036 to 0.040 mm. wide and 0.040 to 0.044 mm. long. The penetration stylet is small, measuring 27 $\mu$ long and 5.5 $\mu$ at the base. The ventral sucker is smaller than the oral sucker. It is spherical to subspherical, measuring from 0.027 to 0.030 mm. wide and 0.028 to 0.030 mm. long. A prepharynx and pharynx are present. There are seven pairs of penetration glands.

The excretory system is Y-shaped. The unbranched stem is present, but very short. The tail varies from 0.017 to 0.021 mm. at the base and is 0.130 to 0.164 mm. long. It is attached to a caudal cavity.

Dragon fly larvae were used for experimental penetration. The encystment took place in the insect larvae as in other species.

## Metacercaria

Cysts are slightly oval, quite clear and with only a few scattered, large, clear globules (Fig. 12). The cuticle is covered with spines. The excretory bladder is somewhat W-shaped or butterfly-shaped, darker at the border and lighter at the centre. Five fresh cysts from dragon fly larvae, under a cover glass, varied from 0.087 to 0.108 mm. and 0.102 to 0.118 mm. in diameter.

## Experimental Definitive Host

Fifty cysts, over a week old, were fed to a pigeon. The faeces of the bird were examined five days later and eggs were found. The bird was killed 11 days after infection and seven parasites were recovered from the posterior portion of the intestine just in front of the caeca.

The natural host is unknown. It developed experimentally in the pigeon and took only about five days to become mature.

# PLAGIORCHIS OTTAWENSIS SP. NOV.

## Adult

The parasites are somewhat elongated and have more or less round extremities (Fig. 4).

The cuticle is covered with spines at the anterior end. Measurement of five specimens, 11 and 21 days after infection, varied from 0.496 to 0.616 mm. in width and 2.072 to 2.237 mm. in length.

The oral sucker is subterminal and is 0.181 to 0.191 mm. wide and 0.184 to 0.202 mm. long. The ventral sucker is at the posterior end of the anterior third of the body. The size is about equal to that of the oral sucker. It is from 0.174 to 0.202 mm. wide and 0.167 to 0.188 mm. long. A prepharynx is present but is very short. The pharynx is well developed, spherical to subspherical. It is four-lobed anteriorly. The oesophagus could not be traced. The intestinal caeca bend slightly forward then turn backward, ending a short distance from the posterior end of the body.

The genital pore is in front of the ventral sucker and slightly to the left of the midline. The ovary is subspherical to somewhat triangular and about the same size as the ventral sucker. It is situated a short distance behind the ventral sucker and slightly to the right of the midline. It varies from 0.167 to 0.195 mm. wide and 0.184 to 0.233 mm. long. The uterus reaches the posterior end of the body beyond the vitellaria, where it loops to accumulate into a large mass. A metraterm is present. The shell gland lies immediately behind the ovary and fills the space between the ovary and the anterior testis. The vitellaria are well developed, with very large follicles. They extend from the region between the genital pore and the middle of the ventral sucker to a short distance from the posterior end, beyond or in front of the ends of the caeca. The follicles occupy the lateral fields of the body, but overlap the caeca. The follicles meet dorsally at the post-testicular region and occupy most of the space.

The egg is operculated (Fig. 17d). It varies from 0.021 to 0.023 mm. wide and 0.036 to 0.038 mm. long. The testes are subspherical and lie obliquely to each other but with the margins in contact or almost in contact with each other. The anterior testis is about the same size as the ovary and at a short distance from it. It is slightly to the left of the midline. The size varies from 0.174 to 0.202 mm. and 0.181 to 0.226

mm. in diameter. The posterior testis is slightly larger, from 0.184 to 0.216 mm. wide and 0.188 to 0.253 mm. long. The ovary and the two testes occupy the middle third of the body. The cirrus sac is long and slightly curved, lying dorsal to the ventral sucker. Its posterior portion extends toward the left with its base behind the middle of the ovary, but never extends beyond the posterior margin of the latter. In a fresh specimen, under a cover glass, it measures 0.112 mm. in maximum width and about 0.775 mm. in length. The seminal vesicle is constricted into a subspherical anterior portion measuring 0.049 mm. wide and 0.059 mm. long, an elongated posterior portion measuring 0.063 mm. wide and 0.202 mm. long. Prostrate glands are well developed. The cirrus is long and protrusible.

The excretory system was studied in detail from a fresh young specimen from a pigeon, four days after infection. The system is Y-shaped as is the case of other species (Fig. 5). It consists of a small bladder and a long unbranched stem reaching the space between the two testes. The two common collecting tubes, after receiving an anterior and a posterior collecting tube on each side become dilated at the level of the posterior margin of the ovary and discharge into the common collecting tube. The flame cell formula is $2((3+3+3)+(3+3+3))$.

Cercaria

Cercaria were obtained from naturally infected snails. When the cercariae were put under a cover glass with plenty of water they tended to swim. The cuticle is covered with spines on the anterior end. The body has no large, clear globules (Fig. 9), but is coarsely granulated and dark in colour. The number of cephalic glands was difficult to determine, but appear to be between seven and nine pairs. The length and the width of the cercariae are approximately in inverse proportion to the state of contraction. Measurement of 30 formalin-fixed cercariae varied from 0.070 to 0.104 mm. wide and 0.219 to 0.308 mm. long. The tail varies from 0.024 to 0.029 mm. wide at the base and 0.175 to 0.220 mm. long. It is attached to a ventral depressed area where no comb-like striations were seen.

The oral sucker is oval and subterminal. It varies from 0.041 to 0.049 mm. wide and 0.047 to 0.056 mm. long. The penetration stylet is about

31 $\mu$ long and 6 $\mu$ wide at the base, which in some instances showed swelling on both sides. The ventral sucker is spherical to subspherical, 0.030 to 0.035 mm. wide and 0.031 to 0.037 mm. long. A prepharynx is present. The pharynx is also present. An oesophagus could not be traced.

The excretory system is Y-shaped with very thick walls. The unbranched portion of the common collecting tube is very short.

Dragon fly larvae were used for experimental penetration. Encystment took place as in other species.

Metacercaria

The metacercaria is very clear. It is spherical to subspherical (Fig. 13). One-day-old cysts were smaller. This indicates that development was taking place. Fifty living cysts from experimentally infected dragon fly larvae measured from 0.129 to 0.146 mm. and 0.129 to 0.143 mm. in diameter. The excretory bladder is tri-lobed and dark, filled with dark granules. The cuticle is covered with spines.

Experimental Definitive Hosts

The natural host is unknown. Experimental infections were made with pigeons, white mice, and chickens. Three hundred and 100 cysts were fed to two mice, respectively. The mice were killed ten and four days, respectively, after infection. The results were negative. About 300 cysts were fed to a chicken which was examined ten days later; six parasites were recovered from the caeca. The parasites were mature and with only a small number of eggs. The size of these specimens is much smaller than those in the pigeons and the vitellaria are only weakly developed.

About 1,000 and 130 cysts were fed to two pigeons, respectively. They were examined 22 and 11 days, respectively, after infection. Thirty-one adults were recovered from the intestine of the first pigeon and seven adults from the second.

Miracidium and Sporocyst

The adult parasites recovered from a pigeon on the 11th August, and the eggs washed from the faeces of the same bird were kept in petri dishes with water which was changed frequently.

Observations for miracidia were made from time to time by examining the dead worms and the eggs. On the 20th September, although no moving miracidia were seen, experimental infection was decided on and four lots of laboratory-bred Stagnicola palustris were infected as follows:

(1) About 50 snails were placed together with the eggs from the faeces of the pigeon and another 30 snails with the dead worms. The snails were allowed to feed on the eggs and the fragments of the dead worms for a few hours. Thirty-four snails remained alive for examination from the 7th October to the 18th November, and all of them gave negative results.

(2) A number of snails was placed on the fragments of the dead worms and the eggs on the same day. Ten snails remained alive for examination between the 7th October and the 16th October. All were negative.

(3) Seventeen snails were placed with the eggs from the faeces of the pigeon on the 21st September. Eight snails remained alive for examination on the 15th October. All were negative.

(4) Twenty-snails were placed with eggs from the faeces of the pigeon on the 20th September.

Twelve snails remained alive for examination. Three were examined on the 6th October and all were negative. Eight were examined on the 4th November. One was found infected with young sporocysts. Another snail was examined on the 11th November and sporocysts were found; some of them contained motile cercariae. Another snail was examined on the 29th November and was also found infected.

The sporocysts are colourless and tubular (Fig. 15). But the shape varies considerably. The cyst wall is apparently very elastic. The diameter depends upon the number of cercariae accumulated at a particular portion. Sometimes a cyst may appear club-shaped, an oval sac with a stalk. The size of a few preserved sporocysts varies from 0.142 to 0.260 mm. wide and 0.950 to 1.250 mm. long. One fresh sporocyst was 0.210 mm. wide and 1.970 mm. long.

The entire life history was completed in the laboratory. The time for the development of the miracidium was not determined. It took about 50 days for the sporocyst to produce cercariae and about 11 days or less for the adult to become mature. The natural host might be a bird, as it was recovered experimentally from both the pigeon and chicken.

## DISCUSSION

*Plagiorchis stagnicolae* is similar to *P. potanini* Skrjabin (1920), and *P. massino* Petrov and Trichonov (1927), but differs from them in the following respects.

In *P. stagnicolae*, the diameter of the cirrus sac is only about half the diameter of the ventral sucker, it is more curved and does not reach the posterior margin of the ovary; the vitelline follicles are thickly set; the eggs are larger (33 to 38 $\mu$ by 21 to 23 $\mu$); and the posterior testis is more forward in position. In *P. potanini*, the diameter of the cirrus sac is more than half the diameter of the ventral sucker, it is only slightly curved and reaches the posterior margin of the ovary; the vitelline follicles are loosely set, the eggs are smaller (32 x 18 $\mu$) and the posterior testis is more backward in position.

In *P. massino*, the vitellaria originate more backward in position; the ovary is median; the cirrus sac reaches the posterior border of the ovary.

Accordingly, it is regarded as a new species and the name *Plagiorchis stagnicolae* is proposed for it.

Schulz and Skworzov (1931) have divided the species of this genus into the sub-genera <u>Plagiorchis</u> and <u>Multiglandularis</u>, characterized by the location of the vitelline follicles. In <u>Plagiorchis</u> they are quite separate, whereas in <u>Multiglandularis</u> they meet dorsally in front of the ventral sucker. In one of the writer's specimens the vitellaria extend across the body in a continuous band whereas in the other 17, they are discontinuous. This suggests that the characters separating these two sub-genera may not be good ones.

<u>Plagiorchis palustris</u>, as mentioned earlier in this study, is the name assigned to this new species. It also most closely resembles <u>P. potanini</u> and <u>P. massino</u> in shape and in the arrangement of the reproductive glands. It differs, however, in several respects.

In <u>P. palustris</u>, the vitelline follicles in front of the ventral sucker are never seen to meet dorsally and are thickly set; the cirrus sac does not reach the posterior border of the ovary; the posterior testis is more anterior in position; and the eggs are larger (35 to 39 $\mu$ by 20 to 23 $\mu$).

In *P. potanini* the vitelline follicles in front of the ventral sucker meet dorsally and are loosely set; the cirrus sac is proportionately much more slender, reaching the posterior margin of the ovary; the eggs are smaller ($32\mu$ by $18\mu$); and the posterior testis is more posterior in position.

In *P. massino*, the vitelline follicles in front of the ventral sucker meet dorsally; the ovary is median; and the cirrus sac is proportionately longer, reaching the posterior border of the ovary.

The adult of *P. palustris* is almost identical with that of *P. stagnicolae* both in size and the relative proportion of different organs. However, it differs in several minor characters. The ovary of *P. palustris* is larger than the anterior testis; the cirrus sac is much broader; the intestinal caeca arch forward at the sides of the pharynx; and the posterior testis is more anterior in position. In *P. stagnicolae* the ovary is slightly smaller than the anterior testis; the cirrus sac is narrower; the intestinal caeca do not arch forward; and the posterior testis is slightly more posterior in position.

There are, however, distinctive characters in the larval stages. In *P. palustris*, the cercaria has a conspicuously larger penetration stylet, 38 $\mu$ by 7 $\mu$; there are tooth-like structures in the caudal cavity; the unbranched portion of the excretory system is fairly long and the body is full of large globules. The metacercaria is oval-shaped and much larger. In P. stagnicolae, the cercaria has a much smaller penetration stylet, 31 $\mu$ by 6.0 $\mu$; there are no tooth-like structures in the caudal cavity; the unbranched portion of the excretory system is absent, and the body is much more transparent. The metacercaria is subspherical and much smaller.

For these reasons, they are described as separate species.

P. canadensis is also similar to P. (M.) potanini Skrjabin (1920), but differs from the latter in several respects. The ventral sucker in P. canadensis is more anterior in position (one quarter to one-fifth the body length from the anterior end); the posterior testis is more posterior in position (about one-sixth the body length from the posterior end); the cirrus sac is more curved; the ovary is slightly to right of midline, and the eggs are larger (38 to 42 $\mu$ by 21 to 24 $\mu$). In P. potanini, the ventral sucker is about one-third the body length from the anterior end; the posterior testis is less than one-fifth the body length from the posterior end; the ovary is median; the cirrus is only slightly curved; the eggs are 0.032 by 0.018 mm.; and the vitelline follicles are loosely set.

Therefore, a new name, P. canadensis, is assigned to it. This species would be included in the subgenus Multiglandularis.

Plagiorchis ottawensis is similar to P. (P.) maculosus var. maculosis (Rud., 1802) Braun, 1902, and P. (P.) maculosus var. citelli Schulz, 1931, in the relative proportion of the different organs. It also resembles P. (P.) maculosus var. anatinus Skrjabin (1927) in the arrangement of the organs.

In P. maculosus the vitellaria originate at the level of the pharynx; the ovary is much smaller than the testes and the ventral sucker, and the uterus does not fill the body caudal of the caeca, whereas in P. ottawensis, the anterior limit of the vitellaria is more posterior in position, at the level between the genital pore and the middle of the ventral sucker, and the uterus reaches the posterior end of the body beyond the vitellaria; its loops accumulate into a large mass and the ovary is slightly smaller than the testes and is about the same size as the ventral sucker.

P. maculosus var. citelli has a pear-shaped cirrus sac. The diameter of its base is about equal to that of the ovary and extends beyond the posterior margin of the latter. The vitellaria are extra-caecal. But in P. ottawensis, the cirrus sac is much narrower, is not pear-shaped and its width is much less than the diameter of the ovary. The vitellaria overlap the caeca and occupy the inter-caecal area behind the testes.

In P. maculosus var. anatinus, the cirrus sac is very slender and its length is less than twice the diameter of the ventral sucker. The testes are very large being much larger than the ovary. In P. ottawensis the cirrus sac is broader and longer. The testes are only slightly larger than the ovary.

In addition, all three varieties of P. maculosus are proportionately broader than the writer's specimens. Therefore, a new name, P. ottawensis is assigned to it.

Plagiorchis is a very large genus of trematodes with a great range of hosts which are found in every group of animals including man (Africa and Garcia, 1937; Sandground, 1940). It appears that this genus is still in the process of adaptation, or is an easily adaptable parasite giving rise to numerous varieties. This makes systematic studies very difficult.

It is probable that the number of species of this genus should be reduced. In the present study, for example, there is a close similarity between the adults of P. stagnicolae and P. palustris, but there are some distinct differences between the larval stages, thereby giving the writer the impression that any attempt to reduce the number of species should be supported by the larval characters, as minor adult characters might often be overlooked.

AFRICA, C. M. and GARCIA, E. Y.

1937 *Plagiorchis* sp. a new trematode parasite of the human intestine. Papers on Helminthology: 30th Jubileum of the activities of K. J. Skrjabin, Moscow, pp. 9-10.

BUTTNER, A.

1950 Première démonstration expérimentale d'un cycle abrégé chez les trématodes digénétiques. Cas du *Plagiorchis brumpti*. Annales de Parasitologie 25 : 21-26.

CORT, W. W. and AMEEL, D. J.

1944 Further studies on the development of the sporocyst stages of Plagiorchiid trematodes. J. of Parasitology 30 : 37-56.

CORT, W. W. and OLIVIER, L.

1943 The development of the larval stages of *Plagiorchis muris* Tanabe, 1922, in the first intermediate host. J. of Parasitology 29 : 81-99.

FREITAS, J. F. T. de.

1941 Sobre Alguns Trematódeos parasitos de Rãs. Rev. Brasil. Biol., 1 : 31-40.

LENT, H. and FREITAS, J. F. T. de.

1940 Sur la position systématique de *Distoma arrectum* Molin, 1859. Annaes da Academia Brasileira de Sciencias 12 : 319-323.

McMULLEN, D. B.

1937 The life histories of three trematodes, parasitic in birds and mammals, belonging to the genus *Plagiorchis*. J. of Parasitology 23 : 235-242.

MEHRA, H. R.

1937 Certain new and already known distomes of the family Lepodermatidae Odhner with a discussion of the family. Z. Parasitenk. 9 : 429-469.

OGATA, T.

1938 Contribution à la connaissance de la Faune Helminthologique Coréenne. I. Une nouvelle espèce de trématodes provenant de chauves-souris. Annot. Zool. Japan. 17 : 581-586.

OLSEN, O. W.

1937 A systematic study of the trematode subfamily Plagiorchiinae Pratt, 1902. Trans. Am. Micro. Soc. 56 : 311-339.

PARK, J. T.

1939 Trematodes from Mammalia and Aves. II. Two new trematodes of Plagiorchidae: Plagiorchidaes (Plagiorchoides) rhinolophi n. sp. and Plagiorchis orientalis n. sp. from Tyôsen (Korea). Keizyô. J. of Med. 10 : 1-6.

PARK, J. T.

1939 Trematodes from Mammalia and Aves. III. A new trematode of the family Plagiorchidae Ward, 1917, Plagiorchis magnacotylus sp. nov. Keizyô J. of Med. 10 : 43-45.

SANDGROUND, J. H.

1940 Plagiorchis javensis n. sp. a new trematode parasitic in man. Rev. Med. Trop. Parasit. Habana 6 : 207-211.

SOBOLEV, A. A.

1946 (Three new species of trematodes in Wading Birds). Collected papers on Helminthology dedicated by his pupils to K. I. Skrjabin in his 40th year of scientific, educational and administrative achievement. pp. 247-251.

SOLTYS, A.

1949 Pasożyty wewnętrzne drobnych gryzoni leśnych (Muridae) Parku Narodowego w Bialowieży. Annales Universitatis Mariae Curie - Sklodowska. Lublin. 4C : 233-259. (in Polish, English summary pp. 258-259.)

STROM, J. K.

1940 New species of trematode worms of the genus Plagiorchis. Mag. Par. Inst. Zool. Acad. Sc. U.R.S.S. 8 : 225-231. (In Russian, English summary, pp. 230-231.)

STROM, J. K.

1940 On the fauna of trematode worms from wild animals of Kirghisia. Mag. Par Inst. Zool. Acad. Sc. U.R.S.S. 8 : 189-224. (In Russian English summary, pp. 219-224.)

TANG, C. C.

1941 Contribution to knowledge of helminth fauna of Fukien. Peking Natural History Bulletin, 15 : 299-316.

YAMAGUTI, S.

1939 Studies on Helminth fauna of Japan. Part 25. Trematodes of Birds. IV. Jap. J. Zool. 8 : 129-210.

YAMAGUTI, S.

1943 Cercaria of Plagiorchis muris (Tanabe, 1922). Annot. Zool. Japan. 22 : 1-3.

ZERECERO, T. D.

1949 Acerca de una nueva especie del genero Plagiorchis Lühe 1899; en el intestino de Tyrannus sp. (Aves, Passeriformes, Tyrannidae). Anales del Instituto de Biologia, Mexico. 20 : 293-299.

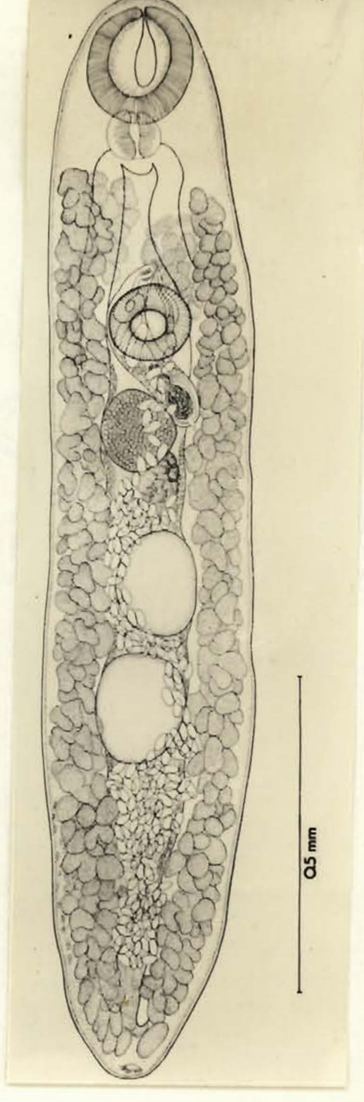



Fig. 1. An adult of Plagiorchis stagnicolae sp. nov. from an experimentally infected pigeon.

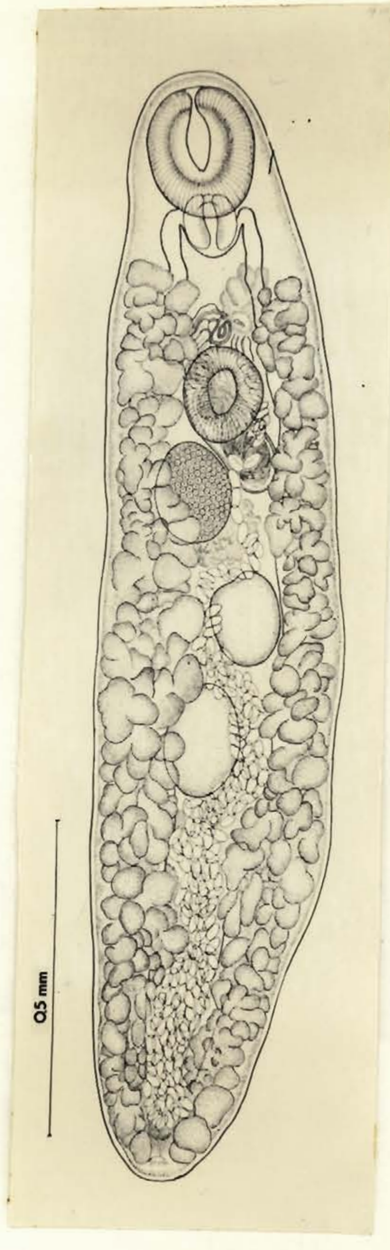



Fig. 2. An adult of Plagiorchis palustris, sp. nov., from an experimentally infected pigeon.

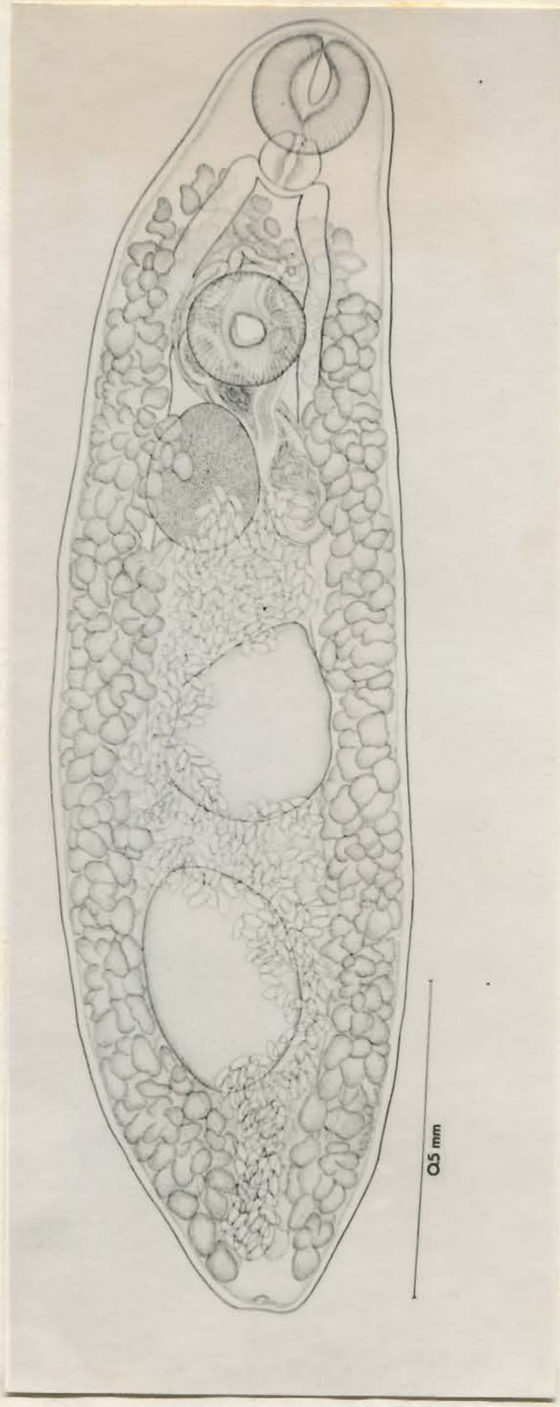



Fig. 3. An adult of Plagiorchis canadensis, sp. nov., from an experimentally infected pigeon.

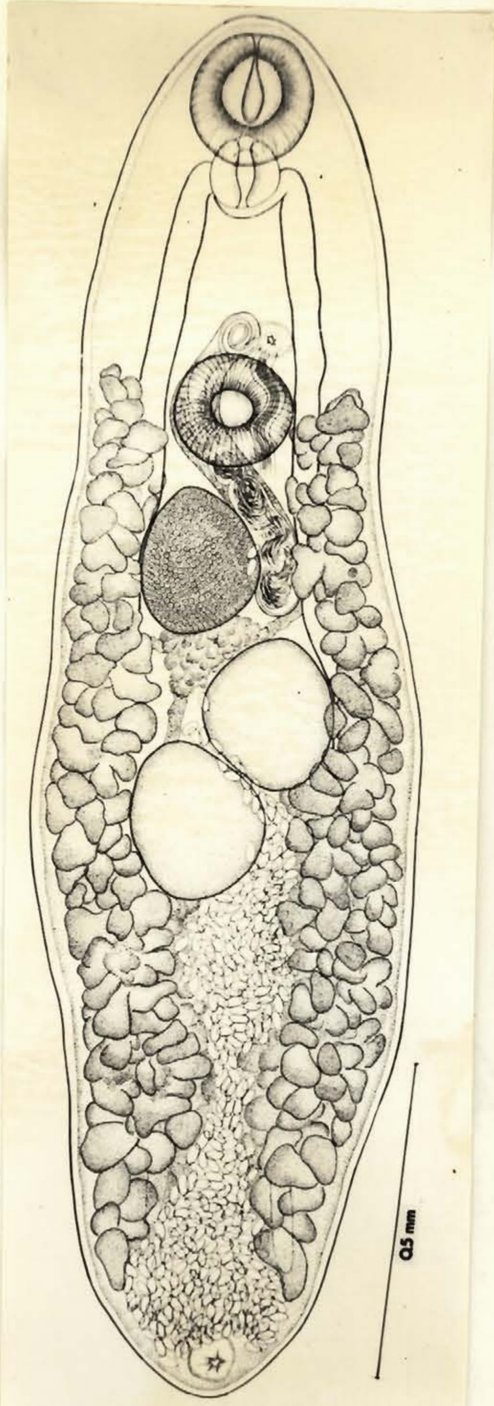



Fig. 4. An adult of *Plagiorchis ottawensis*, sp. nov., from an experimentally infected pigeon.

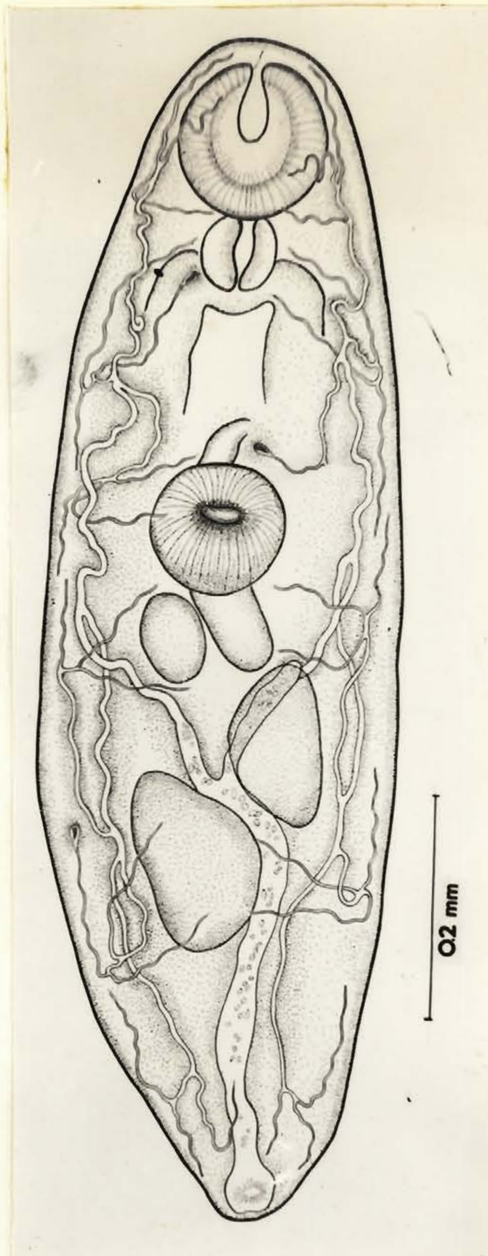



Fig. 5. A young specimen of Plagiorchis ottawensis (4 days after infection) showing the excretory system.

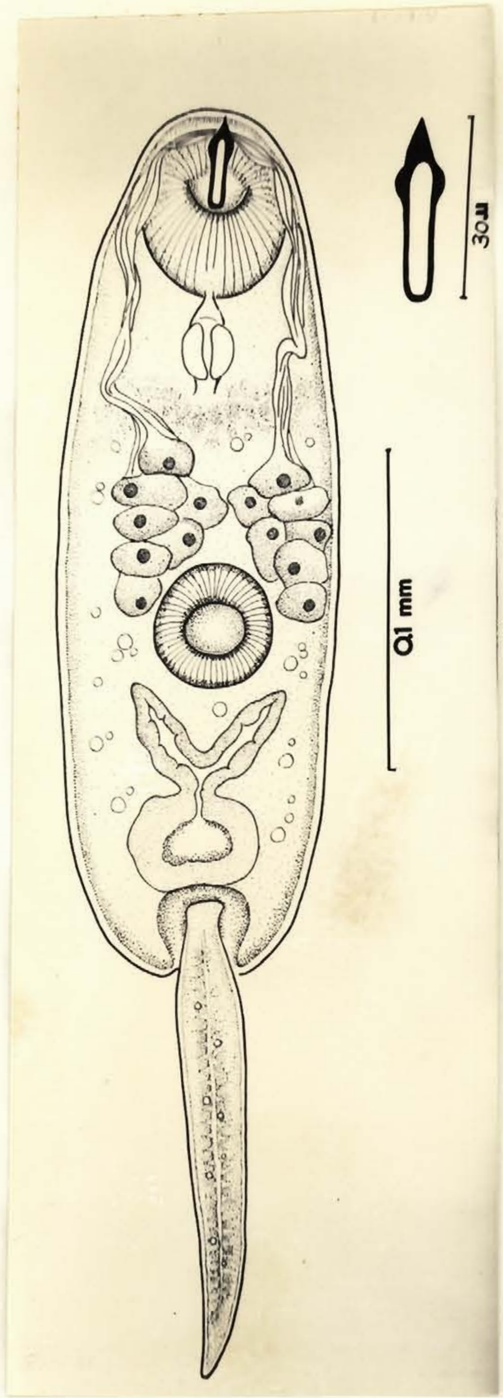



Fig. 6. Cercaria of Plagiorchis stagnicolae

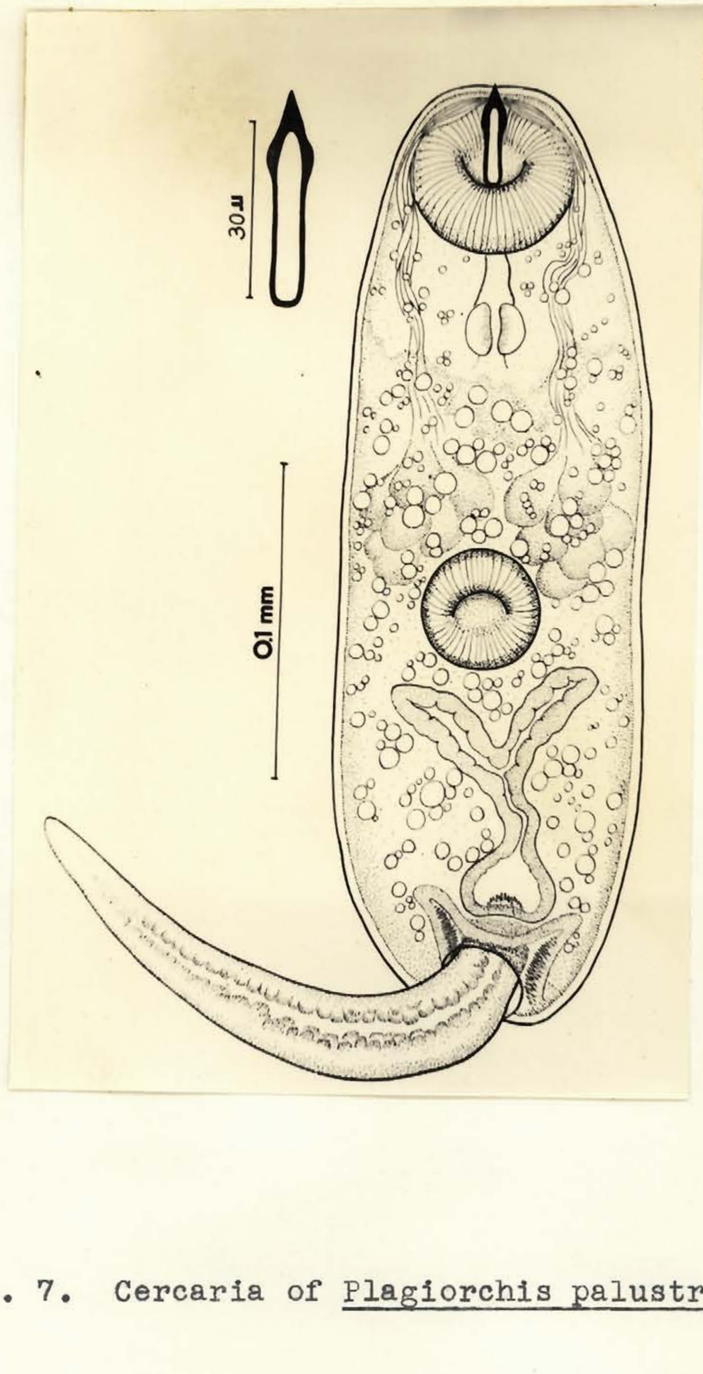



Fig. 7. Cercaria of Plagiorchis palustris

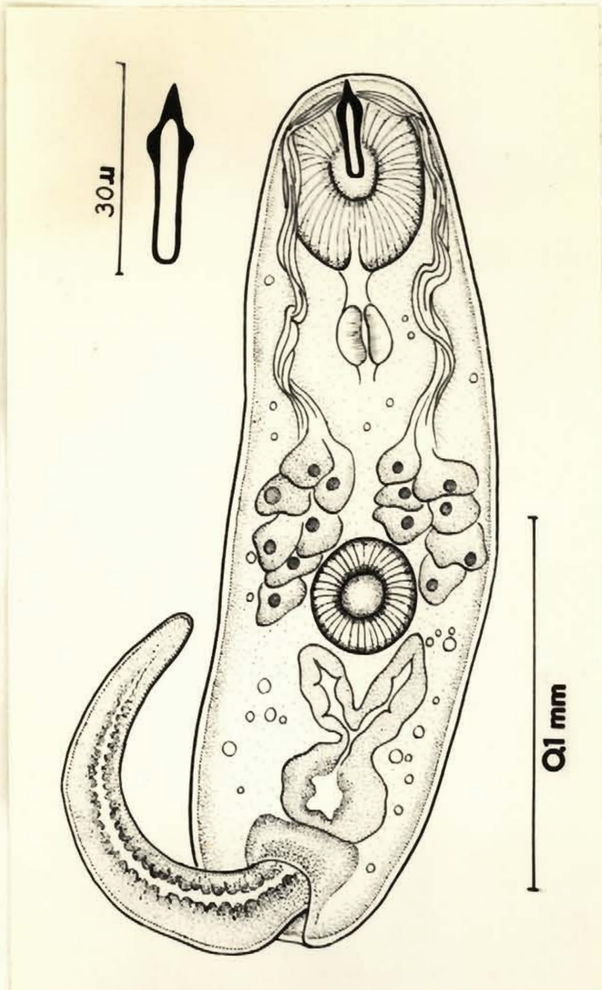



Fig. 8. Cercaria of <u>Plagiorchis canadensis</u>

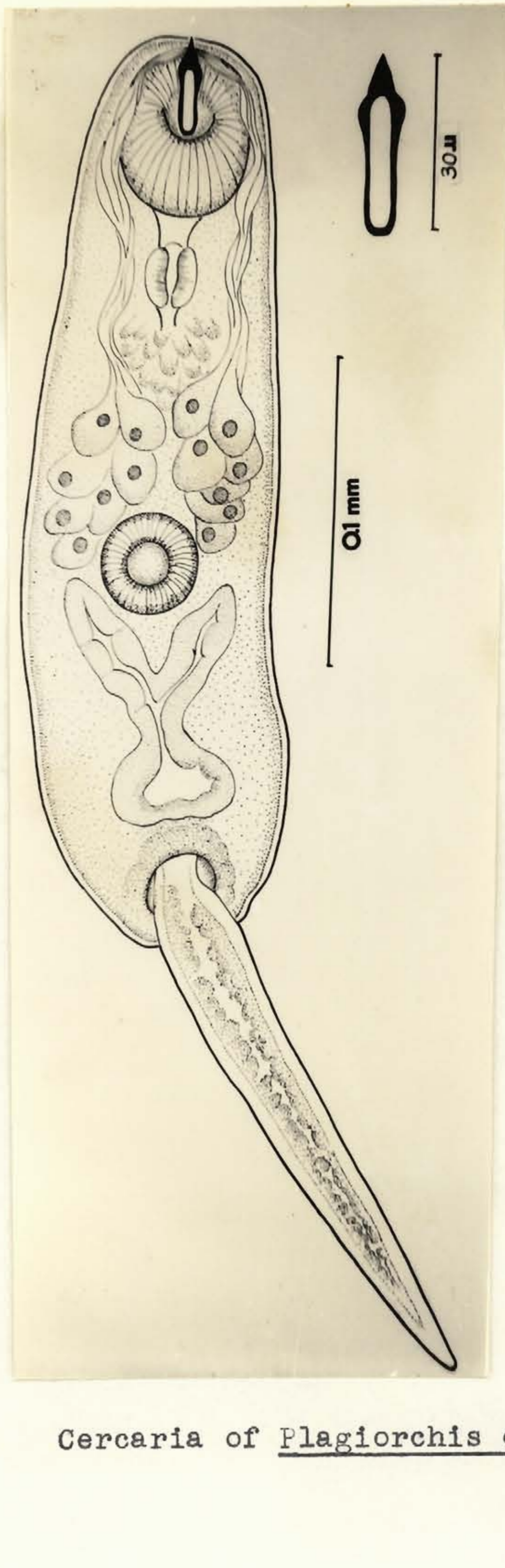



Fig. 9. Cercaria of Plagiorchis ottawensis


Fig. 10. A metacercaria of Plagiorchis stagnicolae

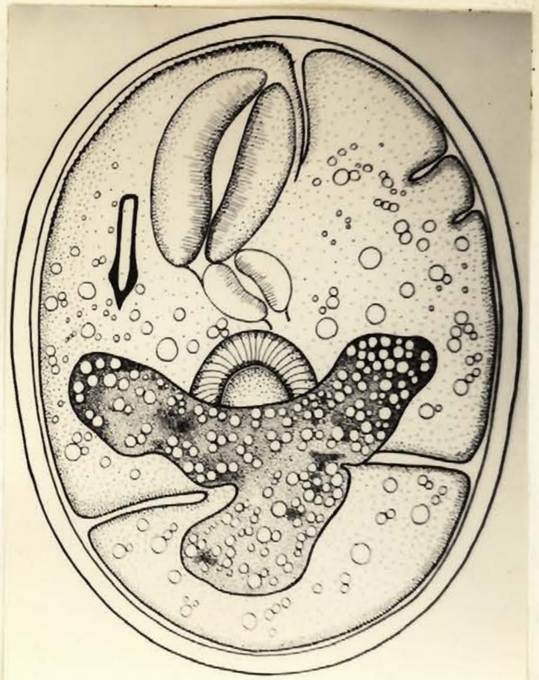


Fig. 11.  A metacercaria of Plagiorchis palustris

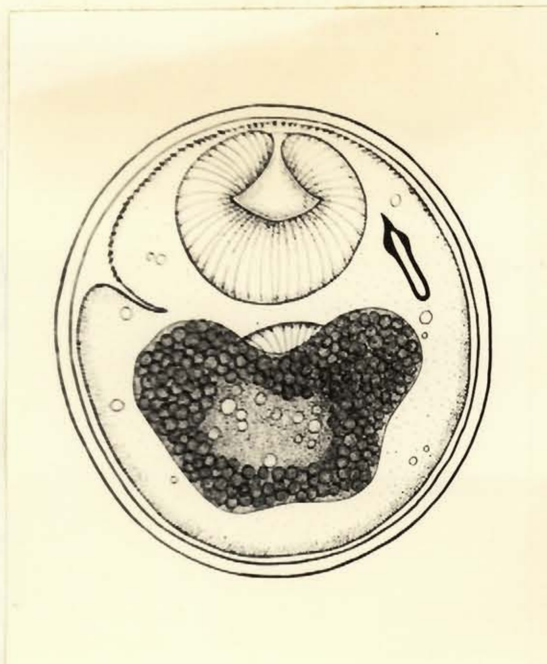


Fig. 12. A metacercaria of Plagiorchis canadensis

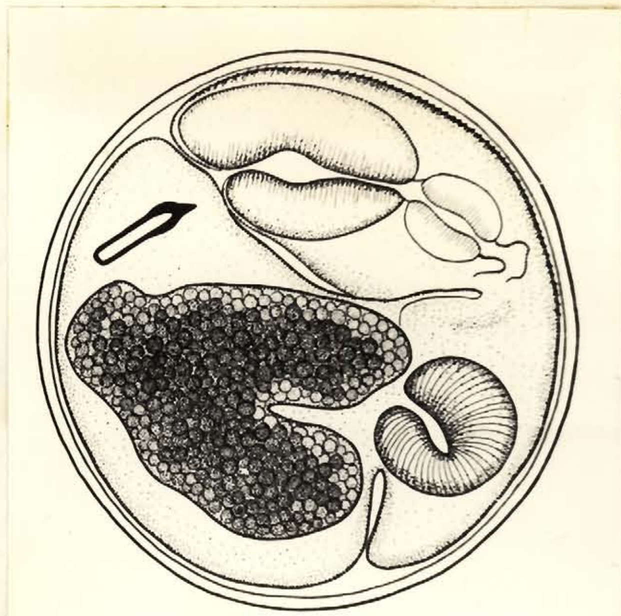


Fig. 13. A metacercaria of Plagiorchis ottawensis

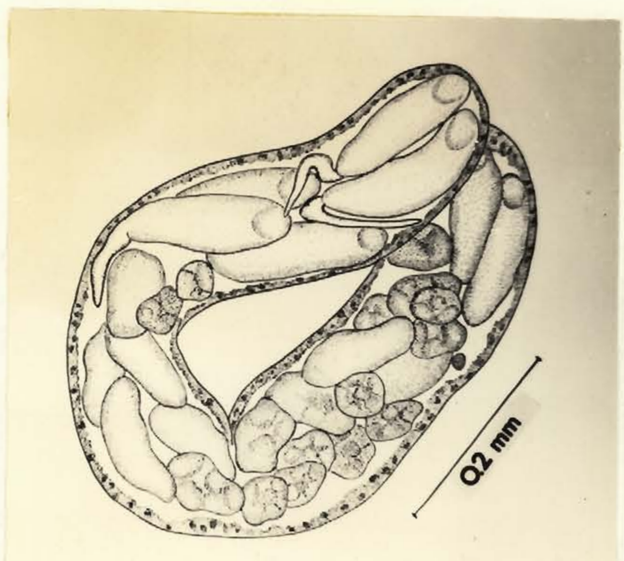



Fig. 14. Mature sporocyst of Plagiorchis stagnicolae from naturally infected snail.

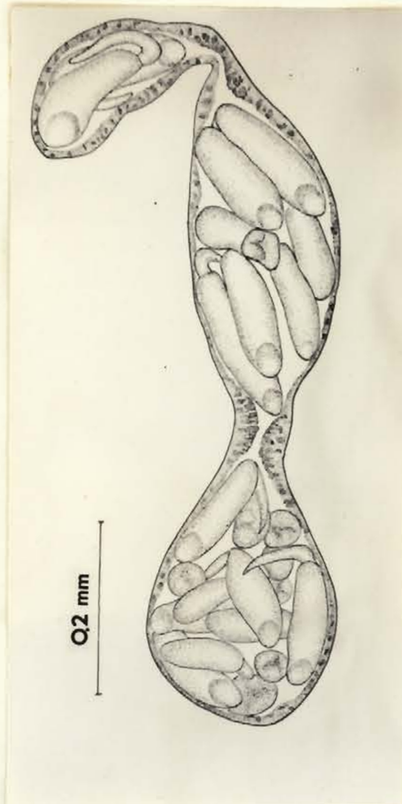



Fig. 15. Mature sporocyst of Plagiorchis ottawensis from naturally infected snail.

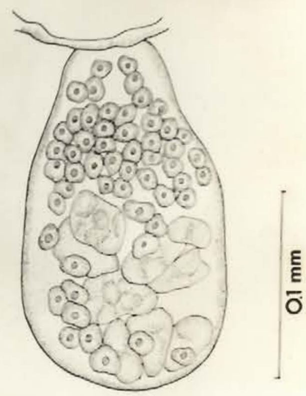



Fig. 16. A young sporocyst of *Plagiorchis ottawensis* from experimentally infected snail, attaching to the digestive tract, showing the germ cells and germ masses.

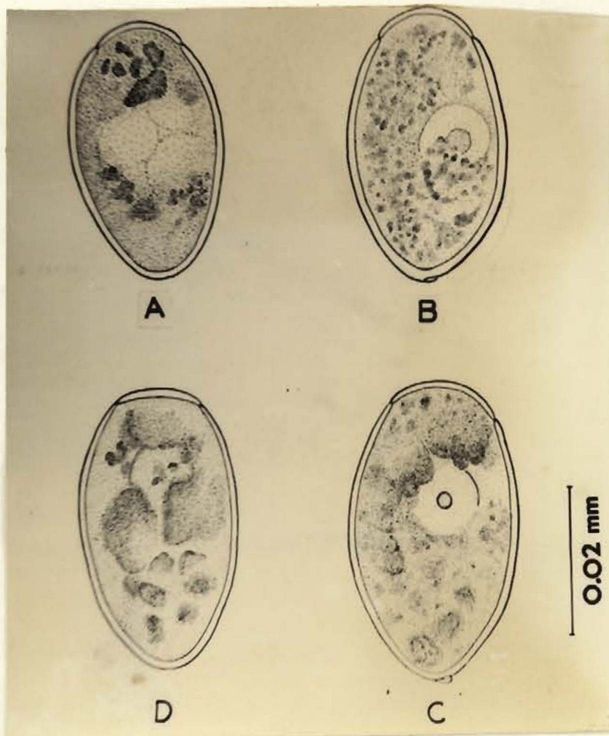



Fig. 17. a - egg of Plagiorchis stagnicolae
b - egg of Plagiorchis palustris
c - egg of Plagiorchis ottawensis
d - egg of Plagiorchis canadensis

## PART III. A STUDY OF THE LIFE HISTORY OF NOTOCOTYLUS STAGNICOLAE HERBER (1942) (FAMILY NOTOCOTYLIDAE)

## CONTENTS

## INTRODUCTION

During the summer of 1951 a monostome cercaria was found in _Stagnicola palustris_ collected from the Ottawa River. The incidence of infection is very low as only two snails were found infected in May and in July.

Experimental infections were attempted and adults were subsequently recovered from chickens. The trematode is very similar to _Notocotylus stagnicolae_ Herber (1942), but differs from it in many minor characters. Therefore, the writer considers it desirable to describe the adult as well as some of the larval stages.

## MATERIAL AND METHOD OF STUDY

The infected snails were kept in separate containers. Some of the cercariae which emerged were removed immediately from the water and fixed in hot formalin for measurement. However, the morphological studies of the cercariae were made from living specimens.

The snail was placed in a petri dish between nine in the morning and midday, during which time, encystment took place on the wall of the container. The petri dishes were then put in a larger container with water and the water was changed every day. The cysts were later removed with a needle for experimental feeding.

The cercariae were also removed immediately after emerging to slides where encystment would take place. The slides were then fixed and mounted.

Chickens, white mice and hamsters were used for experimental infections. The adults recovered from experimental birds were fixed in Bouin and stained with borax carmine and alum carmine.

Adult

The body is elongated, flattened, and concave ventrally (Fig. 1). The ventral surface is covered with spines. Those on the anterior end are scale-like (Fig. 3) and gradually decrease in size toward the middle of the body. The spines on the posterior half of the body are very fine and were seen up to the testicular region. Fine spines were also seen on the anterior half of the dorsal surface.

There are three rows of glands on the ventral surface. From 19 specimens, the number of glands in the lateral rows varies from 16 to 19, being usually 16 to 17.

The number of glands in the middle row is from 14 to 16, usually 15. The most posterior gland in each row is smaller than those in front of it. The most anterior gland in the lateral row is sometimes very small. The ventral glands are irregularly oval with a transverse slit and leaf-like indentations. There is almost always one gland in the middle row anterior to the most anterior glands on the lateral rows. In a few specimens two glands on the middle row are seen to be anterior to the most anterior gland on one of the lateral rows.

The measurements of 10 specimens from chickens, 21 to 31 days after infection, vary from 0.946 to 1.103 mm. wide and 3.889 to 4.323 mm. long. The oral sucker is subterminal and muscular and varies from 0.174 to 0.205 mm. wide and 0.148 to 0.174 mm. long. The oesophagus is narrow. It varies from 0,174 to 0.198 mm. long, but in one specimen it was 0.247 mm. long. The intestinal caeca have lateral indentations.. The caeca bend inward at the anterior border of the testes and end at a short distance from the posterior end.

The genital pore is a short distance behind the intestinal bifurcation. The testes are symmetrically arranged at the posterior end and lateral to the intestinal caeca. They are deeply lobed along their outer margins and there are from six to 11 lateral lobes for each testis (Fig. 2a-d). The size varies from 0.255 to 0.360 mm. wide and 0.465 to 0.616 mm. long. The distance between the testes and the posterior end is 0.210 to 0.225 mm. The vas deferens is found partly in the cirrus sac. The cirrus sac is tubular and slightly enlarged on the posterior half. It varies from 0.139 to 0.167 mm. wide and about 1.066 to 1.291 mm. long. The prostate glands are well developed. The cirrus is very long and protrusible and thickly covered with conspicuous tubercles.

The ovary lies inter-caecally between the testes. It is lobed and smaller than the testes, measuring 0.195 to 0.258 mm. wide and 0.198 to 0.261 mm. long. The ootype is in front of the ovary. The uterus passes forward in numerous, closely packed loops which usually do not extend beyond the outer margins of the caeca. On a few occasion, extra-caecal loops were seen. There are five to eight uterine loops in front of the vitellaria. The metraterm lies to the left of the cirrus surrounded by deeply stained glands. It varies from

0.586 to 0.706 mm. long. The vitellaria are well developed. The follicles extend from just behind the middle of the body to the anterior border of the testes or overlap the anterior portions of the testes. The eggs are oval, each usually with two long polar filaments, one at each end (Fig. 5). Variations were observed on a few occasions. In one case, four filaments were seen at one pole with one on the other pole. On another egg, two filaments were seen on one pole, but none on the other. Twenty-four eggs from the anterior end of the uterus fixed in formalin vary from 0.022 to 0.025 mm. by 0.011 to 0.013 mm. The filaments vary in length from 0.200 to 0.280 mm.

The excretory bladder is immediately behind the ovary and at a short distance from the posterior end. It receives two common lateral collecting tubes at its anterior corners. The two lateral collecting tubes receive, in turn, many lateral branches on their outer margin (Fig. 4). These collecting tubes extend to the region of the oesophagus where they unite. The excretory pore is subterminal.

Cercaria

Nine cercariae (Fig. 6) fixed in formalin and mounted in balsam vary from 0.139 to 0.227 mm. and 0.306

to 0.515 mm. long. The tail varies from 0.038 to 0.060 mm. wide and 0.511 to 0.856 mm. long.

The oral sucker is muscular. At the caudal end there are two short, blunt, tube-like sucking organs for attachment ("locomotive pockets"). There are three eye spots, of which the median is the most anterior. The excretory system consists of two collecting tubes which unite behind the median eye spot with a short projection formed from the union. Therefore, the cercaria should belong to the *Yenchenyensis* group (Faust, 1930) as suggested by Rothschild (1938). These collecting tubes are filled with coarse granules of various sizes. Posteriorly, the collecting tubes empty into the excretory bladder at the anterior corners. The bladder is more or less square and opens posteriorly.

Cercariae began to emerge from the snail host during the late morning, between ten o'clock and noon. They left the snail one after another at short intervals. They progress by whipping the tail in a figure-of-eight. The cercariae are positively phototropic. Encystment usually took place on the lighted side of the container. The time between emerging and encystment is from two to three minutes to one-and-a-half hours, on slides. In a container, encystment took place more promptly, usually in less than half-an-hour. Encystment took place on the wall of the container or on the shell of the snail.

The process of encystment was observed under the microscope. The cercaria rounded up and discharged a secretion rapidly from the body surface as it retracted, leaving a notch indicating the anterior end of the cercaria, (Fig. 7). This secretion formed a broad, thin granular layer adhering to the substratum. In the meantime, it began to rotate. The granules from the two main excretory tubes were being poured rapidly into the excretory bladder and subsequently discharged to the exterior as it rotated actively back and forth. These granules were dissolved rapidly in the first few minutes (less than ten minutes), so that a more transparent, thin outer layer of the cyst wall was formed. The rate of dissolving of these granules was gradually reduced as the process proceeded. Many of the granules were only partially dissolved and became embedded in the newly formed inner layer of the cyst wall. In addition to those more or less square granules from the excretory system, there were rod-shaped granules secreted from the parenchymatous cells of the body.

They were also seen embedded in the newly formed cyst wall; these rod-shaped granules have never been seen rotating at the same time as the granules from the excretory system. Together, these granules gave a brownish, yellow colour to the cyst wall. The maximum thickness of the cyst wall was formed in about half-an-hour and there was no increase of thickness seen during the hour the observation was continued. The rotation and discharge of granules gradually slowed down at the end of the process.

Metacercaria

The metacercariae are subspherical (Fig. 7). Seventeen metacercariae, encysted on slides and subsequently stained and mounted, varied from 0.157 to 0.195 mm. by 0.167 to 0.198 mm. in diameter. This measurement includes only the real cyst wall; the outer adhesive layer is not included. The outer cyst wall is more transparent and thinner, being about 10 µ. This measurement was made on a cyst which had been under a cover glass during the whole process of cyst formation. The diameter of the cyst, including the real cyst wall, measured under such conditions, is much larger (0.253 mm.) than in uncompressed specimens.

Experimental Definitive Host

Two young white mice, two young hamsters, and ten one-to-seven-day old chickens were experimentally fed with cysts of from two to a few weeks old. Mice and hamsters all gave negative results. Adults were recovered only from chickens but the number of parasites from each bird was never very large. Ten parasites is the largest number recovered from a chick fed with 120 cysts.

The caeca of the parasites were filled with red blood cells, which gave the digestive tract of the parasites a bright red colour. Apparently, the parasites fed on the tissues of the host instead of on the lumen.

## DISCUSSION

The adults recovered from chickens while very similar to *Notocotylus stagnicolae* Herber (1942), differ in some small respects. They are larger (21 to 31 days old) than those of *N. stagnicolae* (13 days old), but the ovary is smaller than or nearly equal to that of *N. stagnicolae*. The oral sucker and the oesophagus are longer, the testes are larger, and the number of uterine loops in front of the vitellaria is greater. There is a tendency to have one more ventral gland in each row and the shape of these glands appears to be quite different from that figured by Herber.

The formation of the adhesive layer of the cyst invariably leaves an indentation indicating the anterior end of the cercaria. This indentation is not shown in the figure by Herber.

These differences, however, do not appear to be sufficiently great to justify the creation of a new species.

HARWOOD, P. D.

1939 Notes on Tennessee Helminths. IV. North American trematodes of the subfamily Notocotylinae. J. Tenn. Acad. Sci. 14 : 332-340.

HERBER, E. C.

1942 Life history studies on two trematodes of the subfamily Notocotylinae. J. Parasit. 28 : 179-196.

ROTHSCHILD, M.

1938 Notes on the classification of cercariae of the super-family Notocotyloidea (Trematoda), with special reference to the excretory system. Novitates Zool. 61 : 75-83.


Fig. 1. Notocotylus stagnicolae Herber (1942)

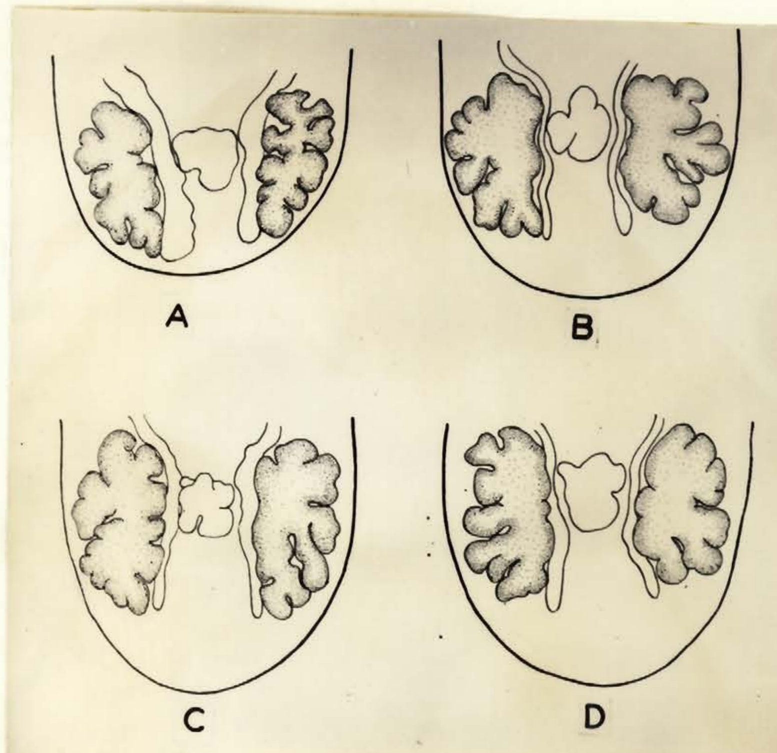



Fig. 2(a-d) Posterior ends of adults showing the ovaries, testes and the caeca.

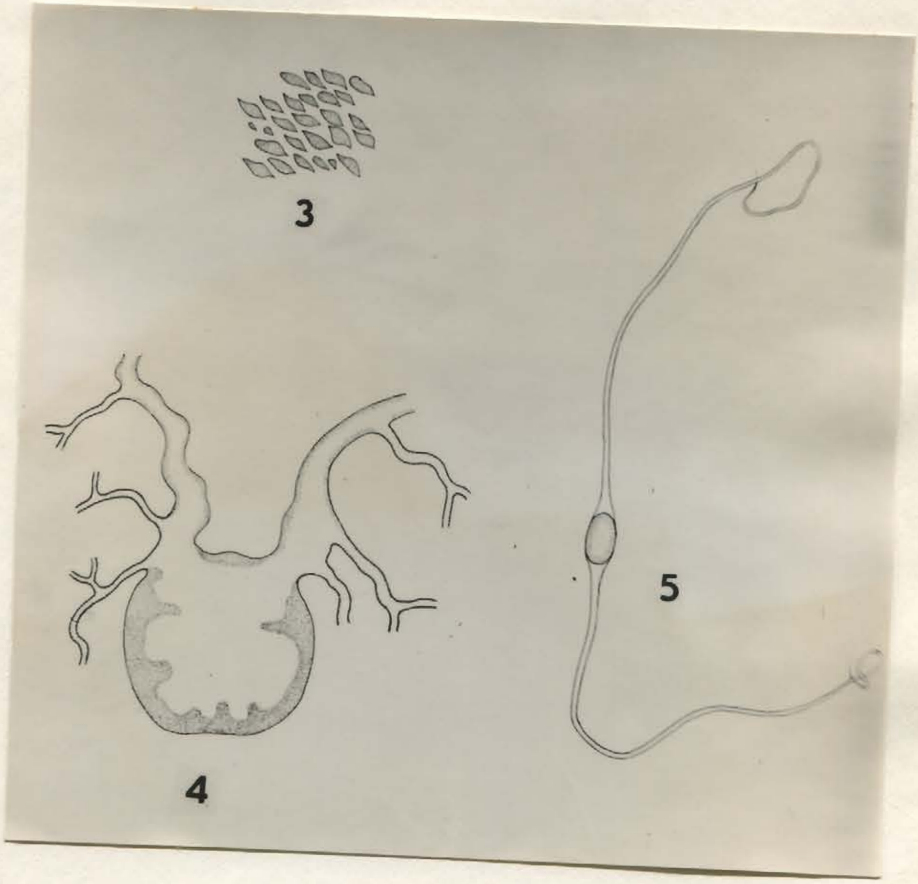



Fig. 3. Scales from the anterior end, ventral surface.

Fig. 4. Excretory bladder and the basal portions of two common collecting tubes.

Fig. 5. An egg.

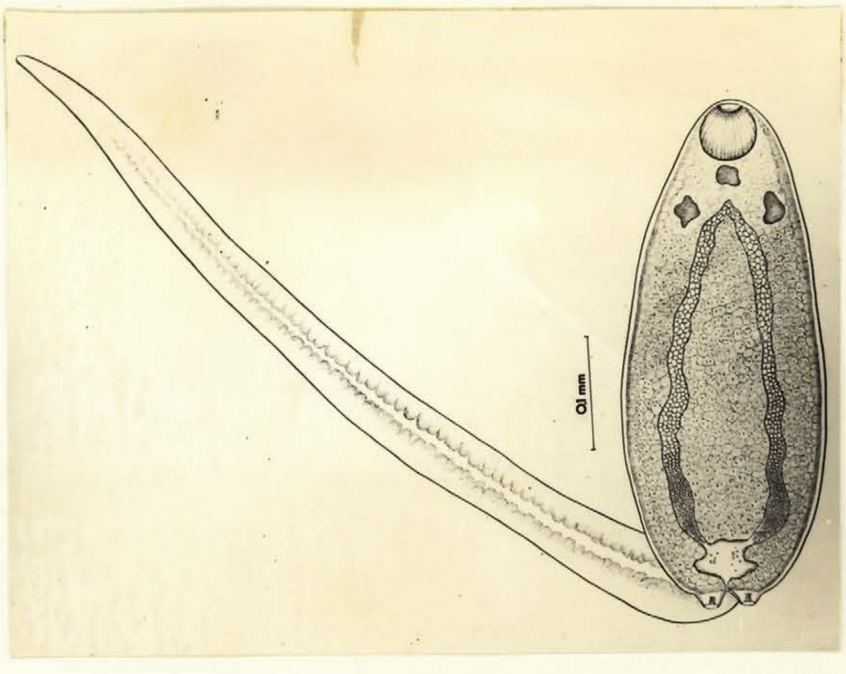



Fig. 6. A cercaria.

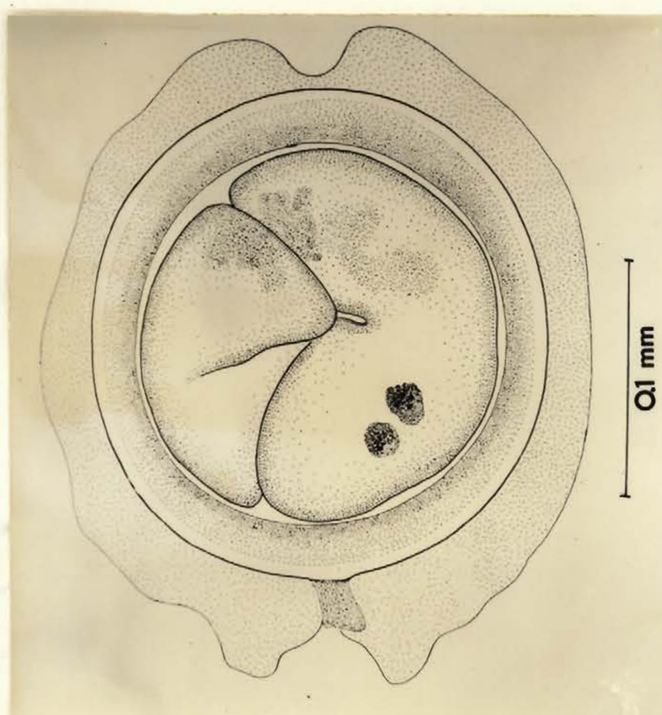



Fig. 7. A metacercaria with its adhesive layer.