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Three new Trematodes found principally in Black Bass.

By

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With Plate 15.

During the spring, summer, and autumn of 1902—1903, a number of black bass from the lakes around Madison were examined for parasites, numbers of which were found to occur abundantly in the different parts of the alimentary tract. Forty-seven bass were examined, all of which contained parasites of some kind, twenty-eight of the number containing one or more of the flukes here described. We have found all three species in the same fish, the most common occurrence, however, being several specimens of *Azygia* and *Leucoruthrus* in the same host, the third and smallest of the three, *Caecicola*, being found in but few fish. The fish examined were taken from Lakes Mendota, Monona, and Wingra, a few being procured from Round Lake, Washburn County, Wisconsin. A very large majority of the fish were the large-mouth black bass, *Micropterus salmoides*, a few specimens only of the small-mouth black bass, *Micropterus dolomieu*, being taken. The parasites were killed in hot water, sublimate or FLEMING'S solution, the last method being used only for small specimens. The large specimens were flattened between two slides and mounted whole or sectioned. The flukes killed in FLEMING'S were not stained; for the others we used alum-carmin, alum-cochineal, alcoholic-cochineal, borax-carmin and haemalum.

Caecincola parvulus n. g. n. sp.

Small distomes (Fig. 1), 0.45 mm \times 0.15 mm, elliptical in outline, the anterior end somewhat flattened, the posterior bluntly pointed. Body entirely covered with minute spines arranged in two series of diagonal rows over entire body. Mouth terminal, oral sucker large; acetabulum much smaller than oral sucker, situated in median line on ventral surface slightly anterior to center; praepharynx and oesophagus quite long, of nearly equal length and width; pharynx well developed; intestinal caeca wide, nearly equal in length to praepharynx, pharynx, and oesophagus combined. Excretory vesicle Yshaped, the median stem about one-third the length of entire worm, the lateral branches extending forward beyond the pharynx. Genital pore median, within anterior margin of acetabulum. Testes are two very large ovoid bodies in posterior half of body, obliquely behind one another; copulatory apparatus absent; vesicula seminis, large and double. Ovary trilobed, at anterior edge of the testes on or slightly to right of median line; yolk-glands lateral, far forward in body; uterus of a descending and an ascending branch, extending to near posterior end of body; receptaculum seminis dorsal to ovary. In caecal tubes and stomach of the wide-mouth black bass, *Micropterus salmoides*.

This small Trematode was generally found in the caecal tubes of the host which part of the alimentary tract would always, in an infected fish, contain more of the parasites than any other part. They were also found in the stomach and upper part of the intestine. The fluke was not noticeable for frequent occurrence but for the great number present in the few infected fish. The parasites could just be seen without a lens, appearing as small, dark specks suspended in the fluid which filled the caecal tubes.

OSBORN (6) describes a new genus of fluke found in the stomach and intestine of the black bass from New York and Michigan. His description and figures at first led us to believe that we had found specimens of the parasite he had described. OSBORN says of this new fluke, *Cryptogonimus chili*, "the worms appear to the naked eye as extremely minute black spots in the yellowish chyle of the host" and he then gives their length as from 0.525 mm to 9.3 mm. The first measurement would correspond very closely to our specimen, the second, would not and we should hold this to be a mis-print, a worm of this length surely appearing larger than "a minute black

spot". There are a number of marked differences between *Cryptogonimus* and *Caecicola* most striking of which is the possession by the former of two ventral suckers and of "eyes". The position and comparative size of the organs within the two flukes is very similar. OSBORN having used the possession of two ventral suckers as one of the chief characteristics of his genus would exclude our specimen, although we can not get rid of a suspicion that the two are identical. *Caecicola* resembles, in its general appearance, the genus *Gymnophallus* of ODHNER (5). This fluke has, however, a simple ovary, a large pars prostatica and the yolk glands near the center of the body. The external form of *Caecicola* is, in live specimens, fairly constant but in those which have been killed and mounted there is apt to be a variation, caused by a constriction of the body in the praepharyngeal region. This is shown to a slight degree in the figure. The entire body is covered with minute spines visible only under a high power. These run diagonally around the body and where the rows cross each other little spineless areas are found; these are nearly square. The mouth is of medium size, the oral sucker, diameter 0.14 mm, is nearly equal to the body at its widest part. The diameter of the acetabulum is 0.05 mm. The mouth leads into a long praepharynx 0.06 mm in length, which passes into a well developed pharynx 0.045 mm in length. The oesophagus follows with a length of 0.035 mm, and is of equal thickness to the praepharynx. Just anterior to the acetabulum the oesophagus passes into the two intestinal caeca which extend slightly beyond the middle of the body reaching the anterior edge of the testes. Each caecum is wide, much wider than the oesophagus, its wall formed of a single layer of large cells. At either side of the praepharynx are a number of large glandular cells which apparently pass forward to open into the mouth region. The cerebral ganglia (Fig. 1, *br*) are very large, one either side of the praepharynx. They are connected by an oesophageal commissure which crosses the praepharynx. The only other traces of the nervous system which we found were two nerves passing forward, one from each ganglion.

The excretory vesicle (Fig. 4) is large and when distended occupies a great part of the body. The excretory pore is terminal, opening into a short, narrow, thick-walled duct which leads into the median stem of the vesicle. This median stem extends forward about one-third the length of the body; it is, when distended, large, but narrower than either lateral stem. The entire vesicle does not

appear separable into a bladder and lateral stems but all parts are more bladder-like and either empty or filled at the same time. When the vesicle is filled it occupies an exceedingly large space within the body, each lateral stem being at least one-third the width of the body often appearing larger than we have figured.

The genital pore is situated just within the anterior edge of the acetabulum, the male opening just anterior to that of the female (Fig. 3). The testes, $0.14 \text{ mm} \times 0.09 \text{ mm}$, are very large, reaching in mature specimens a length equal to one-fifth the length of the fluke. Seen ventrally, they have an oval outline, are situated nearly in the same transverse plane, but one always slightly in advance of the other; proportionally they are similar in size, shape and position as the testes of *Distomum cirrigerum* v. BAER. Counting the relative position of the testes in thirty specimens, we found that fifteen had the right testis slightly in advance of the left, the other fifteen showed the left testes a little in front of the right. From the anterior margin of each testis a vas deferens passes forward and towards the center. The two soon unite and the tube thus formed widens twice, each time into a large ovoid swelling, a seminal vesicle (Figs. 1 and 3, *vs. sem*). This double seminal vesicle varied greatly in size in young and old worms becoming large in mature specimens, especially so when filled with sperms. Both seminal vesicles are near the median line, the anterior one often being partially hidden, in ventral view, by the acetabulum.

The ovary (Fig. 2), is distinctly tri-lobate; its position is median, or nearly so, and ventral to the anterior part of the testes. These three lobes are of equal or nearly equal size, each $0.035 \times 0.027 \text{ mm}$; the median may be slightly larger than either lateral lobe. In some specimens one or, may be, two of the lobes are concealed by the third making the tri-lobate character evident only after a careful examination. An oviduct passes dorsally. In some specimens traces of a shell-gland were seen but we were unable to find any specimen showing it distinctly enough to describe. The receptaculum seminis is large and lies dorsal to the ovary. The yolk glands are situated far forward in the body lying at each side of the praepharynx and pharynx, never extending as far back as the intestinal caeca. Each gland consists of a small number of irregular follicles the separate ducts from which could not be seen. The yolk-ducts pass backwards from the posterior end of the glands, the two ducts meeting slightly anterior to the receptaculum seminis:

at their point of union a small yolk reservoir is formed. LAURER's canal, if present, was not seen. The uterus is not nearly so prominent as in most distomes, its entire length is not very much greater than that of the fluke. It starts from a point near the center of the body in the ventral half and descends on the right side in an irregular, bending manner; before the posterior end of the body is reached it passes to the left side ascending here in a manner similar to that of its descent. In no specimens were the eggs so numerous as to greatly obstruct a view of the other organs.

Leuceruthrus micropteri n. g. n. sp.

Body tongue-shaped, the anterior end blunt, the posterior end pointed (Fig. 8). Length from 4 to 7 mm. Body smooth. Oral sucker well developed ventral in position near anterior end of body; acetabulum one-half the size of oral sucker, median, a little anterior to center of body. Well developed pharynx present; intestinal caeca extend to end of body. Excretory vesicle Yshaped, the median stem extending to the ovary, the lateral branches into anterior part of the body. Genital pore median a little anterior to the acetabulum. Testes, one diagonally behind the other, a little posterior to the acetabulum. Copulatory apparatus present. Ovary median, midway between the testes and the posterior end of the body. Yolk glands lateral in the posterior half of the body. LAURER's canal present. Habitat, mouth and stomach of large- and small-mouth black bass.

In the black bass examined, this distome was found oftener than either of the other two. A large majority of the specimens were taken from the stomach, a few in the mouth or on the gills. It is easily recognized by its peculiar pinkish or yellowish tinge hardly dark enough to be described as red or yellow. It was found in black bass from all three of the Madison lakes and from Round Lake.

The oral sucker is large, 0.75 mm. in diameter and is situated ventral just behind the anterior margin of the body. (All the measurements were taken from a specimen 5 mm long.) The acetabulum, diameter, 0.45 mm, is median, a little in front of the center of the body. The pharynx is large, praepharynx and oesophagus absent. The intestinal caeca extend to the posterior part of the body; each caecum at first extends forward for a short distance before passing backward.

The excretory vesicle is Yshaped, the excretory pore terminal, opening into a short, narrow tube which enters the median vesicle.

This latter part is of moderate length, extending forward as far as the ovary, and from its anterior margin the two lateral branches arise and extend forward. We were unable to follow these branches further than the acetabulum but from their abrupt ending and thickness at the end we believe that they extend further into the anterior region of the body.

In a ventral view, the testes are nearly circular in outline, 0.325 mm in diameter and lie nearly in the same transverse plane. One is always slightly in advance of the other but neither the right nor the left is constant in this respect. Both testes are near the center of the body a little posterior to the acetabulum. The vasa deferentia pass forward dorsal to the acetabulum; they join at the base of the cirrus-sac within which the seminal vesicle is bent upon itself, finally, passing into the ductus ejaculatorius (Fig. 9) which empties into the genital sinus just anterior to the female opening.

The ovary is median, slightly lengthened along the transverse axis of the fluke and is situated midway between the testes and the posterior end of the body. A ventral view shows it lying apparently in the fork of the excretory vesicle. An enlarged view of the ovary (Fig. 12) shows it to be filled with eggs of different sizes, the largest and oldest of which are nearest the entrance of the oviduct, the smallest and youngest near the surface. No definite epithelial layer could be distinguished within the ovary but a layer of slightly flattened cells, each with an ovoid nucleus, formed the wall of the oviduct. The eggs are irregular in shape assuming a more definite form as they become larger until, finally, those near the entrance of the oviduct are all somewhat similar. Each egg (Fig. 12a) contains a large nucleus in which an irregular chromatin network can be distinguished, each also possesses a single nucleole. The oviduct passes forward from the ovary for a short distance and joins the receptaculum seminis, receiving LAUREN's canal, a long, narrow tube the external opening of which is dorsal (Fig. 10). From this point the tube passes through the shell gland which lies a little to the right of the median line. Specimens containing but few eggs have the uterus confined to a space between the intestinal caeca on the sides, the acetabulum in front and the ovary behind. When, however, the eggs have increased greatly in number the outline of the uterus becomes lost and it nearly fills the posterior three-quarters of the fluke (Fig. 14). The vagina enters the genital sinus just posterior to the opening of the ductus ejaculatorius.

Each yolk gland consists of a number of follicles on either side, having a slight appearance of being arranged in two or three irregular rows, none of which extend in front of the acetabulum. The yolk duct from each gland passes toward the median line of the body, the two ducts meeting in a yolk-reservoir which lies anterior to the ovary. Each of the many follicles contains a number of cells in which the yolk particles are seen in different stages of development. The follicle wall is very thin, distinguished only by the small, flattened nuclei. The youngest cells within a follicle are small, each encloses an ovoid nucleus which nearly fills the cell there being but a slight difference in the diameter of the two. In somewhat larger cells the nucleus remains of about the same size, its increase in size being very much less than that of the cell. Within the smallest cells a number of dark spots are seen, irregular in position, but all of about the same size. In larger cells these bodies, the yolk globules, have increased in size, growing larger and larger until they finally almost completely fill the cell, often hiding the nucleus. The largest of the yolk globules are irregular in outline and in prepared specimens appear to contain a number of dark granules (Fig. 11).

The wall of the seminal receptacle is quite thick; seen under a high power the cell boundaries are indistinguishable, the spherical nuclei being arranged at regular distances. Each nucleus contains a single nucleolus and one or more vacuoles.

Azygia loossii n. sp.

This fluke (Fig. 5) is oblong-ovate, the anterior end not so pointed as the posterior. length 5 mm to 6.7 mm, breadth 0.5 mm. Body naked. Oral sucker ventral, at anterior end. Acetabulum ventral and median, slightly nearer the anterior than the posterior end. Pharynx fairly large; oesophagus very short; intestinal caeca reaching to posterior end of body. Excretory vesicle consists of a short, wide bladder situated behind the testes and two narrow, lateral branches. Two testes, one behind the other, in posterior part of the body. Ovary in part or entirely in front of anterior testis. Yolk glands dendritic, lateral, in posterior half of body; uterus between ovary and acetabulum. Genital pore median, a little in front of acetabulum; copulatory apparatus present. Habitat, mouth and stomach of wide-mouth black bass, *Micropterus salmoides*, pike, *Lucius lucius*, and dog-fish, *Amia calva*.

This Trematode belongs, we believe, to the genus *Azygia*, agreeing very closely with the description given by Looss (3). Comparing our specimens with the figure Looss (2) gives of *A. tereticolle* and specimens of this species which we have, we notice several differences between them. In *A. loossii*, the gonads are all nearer the posterior end of the body, their relative positions being, however, very similar. The acetabulum in *A. tereticolle* is further forward than in *A. loossii*, the ovary more in advance of the testis and the excretory bladder narrower and longer.

Azygia loossii was found abundantly in the stomach of the black bass, occurring but seldom in the pike or dog-fish. A few examples were found in the mouth and on the gills. It was taken in fish from the three Madison lakes and from Round Lake. While not so abundant as *Leucercuthrus* the two were generally found in the same fish.

The oral sucker, 0.425 mm in diameter, is nearly as wide as the body; the mouth is smaller and leads directly into a well developed pharynx 0.15 mm in width. The oesophagus, if present, is very short and not readily distinguished, the intestinal caeca appearing to arise directly from the pharynx. The long narrow intestinal caeca extend to very near the posterior end of the body, they are much straighter than shown by Looss (2) for *A. tereticolle*.

A short, narrow duct leads from the terminal excretory pore into the short, broad bladder which does not extend in front of the posterior testis. In all the specimens of this fluke, when living, the bladder was swollen and distinctly seen allowing us to separate this from other flukes by the large, bright swelling which appeared in the posterior end of the body. From the anterior margin of the bladder two long, thin, lateral tubes pass forward into the front region of the body. These we have traced almost to the pharynx. The testes, 0.125 mm in diameter, are situated, one in front of the other, generally, a little diagonally, the posterior being the more constant in its median position. The two testes are separated from each other by a distance not so great as the diameter of either one. The vasa deferentia were seen only for a short distance before they reached the cirrus sac. The seminal vesicle is folded and leads into a short ductus ejaculatorius which has its opening into the genital sinus just anterior to the opening of the vagina.

The ovary, slightly larger than either testis, is ovoid, its largest diameter lying at a right angle to the long axis of the fluke.

A short oviduct passes anteriorly and is joined by LAURER's canal; then, passing forward for a short distance, it turns backward through the shell-gland. The uterus occupies the same position as in *A. tereticolle*, lying between the intestinal caeca and not extending back of the ovary or in front of the acetabulum. The vagina opens into the genital sinus just posterior to the male opening.

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Explanation of plate.

<i>act</i> Acetabulum	<i>po. ex</i> Excretory pore
<i>br</i> Cerebral ganglion	<i>po. gen</i> Genital pore
<i>Can. Lau</i> LAURER's Canal	<i>rep. sem</i> Receptaculum seminis
<i>dt. ejt</i> Ductus ejaculatorius	<i>rsr. rt</i> Yolk-reservoir
<i>in</i> Intestine	<i>sac. cir</i> Cirrus-sac
<i>oa</i> Ovary	<i>si. gen</i> Sinus genitalis
<i>o'dt</i> Oviduct	<i>ut</i> Uterus
<i>oes</i> Oesophagus	<i>vd</i> Vas deferens
<i>or</i> Mouth	<i>vg</i> Vagina
<i>plx</i> Pharynx	<i>vgl. ex</i> Excretory vesicle
<i>pphr</i> Praepharynx	<i>vgl. sem</i> Vesicula seminalis.

All figures drawn with camera-lucida.

Plate 15.

- Fig. 1. *Cuecincola parvulus*, ventral view; the ovary drawn somewhat to one side to show underlying parts. 185:1.
- Fig. 2. The ovary, ventral view. 235:1.
- Fig. 3. A diagram of the reproductive organs, lateral view.
- Fig. 4. The excretory vesicle, ventral view. 185:1.
- Fig. 5. *Azygia loossii*, ventral view. 22:1.
- Fig. 6. A diagram showing openings of reproductive organs.
- Fig. 7. A diagram of the female reproductive organs.
- Fig. 8. *Leucocuthrus micropteri*, ventral view. 30:1.
- Fig. 9. A diagram showing openings of reproductive organs.

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Fig. 10. Diagram of female reproductive organs.

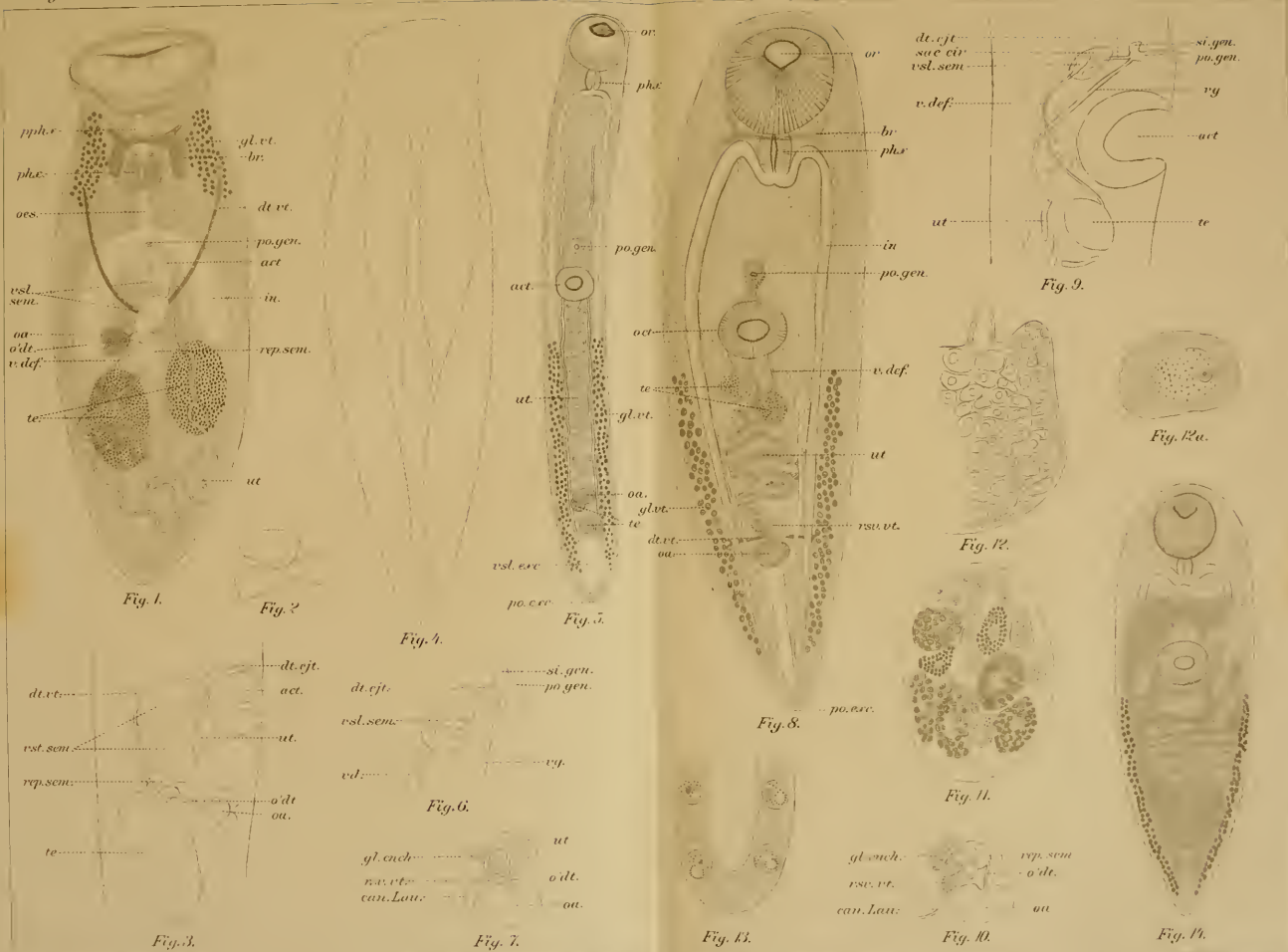
Fig. 11. Single follicle from yolk gland, the enclosed cells showing growth of yolk-globules. 590 : 1.

Fig. 12. Half of ovary. 235 : 1.

Fig. 12a. Single egg from ovary. 720 : 1.

Fig. 13. Part of wall of receptaculum seminis, cut diagonally. 720 : 1.

Fig. 14. Mature specimen with uterus distended with eggs.



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