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ON THE
PARASITES OF THE LAKE FISH.

*I. Notes on the Structure and Life History of Distoma
opacum, n. sp.*

BY

HENRY B. WARD, A. M., PH. D.

FROM THE PROCEEDINGS OF THE AMERICAN MICROSCOPICAL SOCIETY,
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*With the records of
Henry B. Ward*

ON THE PARASITES OF THE LAKE FISH.

I. Notes on the Structure and Life History of Distoma opacum, n. sp.

HENRY B. WARD, A. M., Ph. D.

This work was done while a member of the scientific corps sent out by the Michigan Fish Commission to begin an investigation of the biological conditions in the Great Lakes. The work in 1893 was carried on at New Baltimore, on Lake St. Clair. My thanks are due to the Michigan Board of Fish Commissioners for the many conveniences placed at my disposal in carrying out the investigations on fish parasites, of which this is one result. To Professor J. E. Reighard, director of the party, I owe much for his interest and hearty coöperation in all matters pertaining to this work.

In the first dog-fish (*Amia calva* L.) examined (August 2) the intestine just above the spiral valve yielded a large number (354) of specimens of a small *Distoma*, which is about the size and shape of a mustard seed. It seemed on examination to be a new species, and later study strengthened this opinion. My attention was, however, more particularly directed to it when ten days later I examined a specimen of the same fish which had been brought from Saint Clair Flats, opposite our laboratory. The number of this *Distoma* present was so great as to cover completely the wall of the intestine. They were removed by a pipette to a dish of normal salt solution (0.75 per cent.) and, after examination, preserved in a saturated solution of corrosive sublimate, in Kleinenberg's picrosulphuric acid, and in Flemming's chrom-osmo-acetic acid, about one-third in each. One of these lots counted later showed 1,023 individuals present. The fish just mentioned had enjoyed a meal of *Cambarus propinquus*, 26 of which were still easily recognizable in his stomach. I noticed at once in the crayfish, which had the cephalo-thorax crushed and in most cases its dorsal portion gone, a large number of small cysts, while around these cysts were occasional

free Distomes, and in the stomach were to be found both cysts and free Distomes, whose identity with those in the crayfish and with the specimens from the intestine was afterwards established beyond a doubt. These results were communicated to the Society in a paper which was, however, read by title only.

During the latter part of August the work of examining the fish for parasites was entrusted to Mr. Dwight Lydell, an experienced and careful assistant of the Fish Commission, and from his record of two specimens of *Amia* examined August 31, I take the following: "One with tapeworms was feeding on small fish, the other on crawfish." The parasites from "the other" were this *Distoma*, both free and enclosed in cysts, so that the source of the infection seemed clear.

External Anatomy.

While at rest the *Distoma*, if viewed from above or below, has much the shape of a flattened pear, varying from oval to elliptical, and being usually more pointed at the anterior end (Fig. 1). In lateral aspect the anterior half to three-fifths of the body is much thinner than the posterior portion, and this difference is more striking in the mature specimens, owing to the mass of accumulated eggs. As the worm moves the anterior portion is greatly elongated, often to two or three times its original length, until the animal resembles a gourd, or if turned toward one side, a crooked-neck squash. During movement the posterior part of the body undergoes little or no change in form, but is dragged along like an unwieldy burden.

The living specimen measures about 1.7 mm. in length and 1 to 1.16 mm. in width; the average of ten alcoholic specimens gave 1.62 mm. by 0.83 mm. It is of an opaque white,* with an area of yellowish brown on the posterior portion. The anterior or oral sucker is distinctly subterminal, and its diameter varies from 147 μ in the living specimen to 155 μ in the alcoholic. In form it is circular and its opening is directed ventrad. The ventral sucker or acetabulum is located about at the center of the body. It is slightly larger than the oral sucker, measuring about 210 μ in diameter in the living specimen and but 164 μ in ten alcoholic specimens. The entire surface of the body is free from spines.

*The specific name is taken from this character of the living worm.

Internal Anatomy.

In the alimentary canal extending posteriad from the oral sucker one can distinguish (Fig. 2) several regions: first, a short pharyngeal sac, varying in length from 80μ to 125μ . Just before joining the pharynx it is reflected, making a small circular pocket which varies in size with the position of the pharynx. The latter is a muscular organ, elliptical in shape, measuring on the average 54μ by 40μ , with its long diameter in the chief axis of the body. From it the œsophagus leads directly posteriad about 0.51 mm., and then gives rise to the two clavate crura intestini. The latter are short, measuring only 0.15 mm. in length. The entire alimentary system lies thus in the anterior half of the body in front of the ventral sucker. The long œsophagus and the short crura show this to be a member of Dujardin's subgenus *Brachycœlium*.

The excretory pore at the posterior end leads by a short canal into the excretory reservoir, which is large and is prolonged into two long tips or horns, one on either side. These extend forward as far as the region of the testes (Fig. 3). No observations were made on further details of this system. The reservoir forms one of the most striking features of the adult and can also be seen in the encysted form to be described later.

What was determined of the nervous system may be seen in Fig. 1. The ganglionic mass about the pharynx and some of the nerves branching from it can be identified with ease on almost all specimens. Most prominent are the two nerves which extend posteriad.

The genital pore lies at the left of the ventral sucker and immediately adjacent to it. The slit-like opening, which is 30μ to 35μ in length, leads into a small genital cloaca (Fig. 4), into which the ducts of the sexual organs open. This cloaca possesses overhanging lips, which are commonly nearly in contact.

The male sexual organs are comparatively simple. The two testes lie, one on either side, behind the ventral sucker, and under the coils of the uterus in a mature specimen, so that in many cases they can be demonstrated only in sections or, at most, as an indefinite area of deeply stained tissue below the egg masses. In those individuals in which the production of eggs has not reached its height one can see clearly both the testes and the fine vasa efferentia. The latter, passing forward toward the center of the body, lie above the ventral sucker, above or just in front of which they join

at the point at which they empty into the enormous vesicula seminalis. In stained specimens this is a prominent object in front of and partly dorsal to the acetabulum; it occupies chiefly the left side of the body, and ordinarily is distended with a mass of spermatozoa. Toward the left side of the organ it is drawn out to a point, from which the ductus ejaculatorius passes almost directly ventrad to its opening into the genital cloaca. The duct has a diameter of about $10\ \mu$, with a total length of 0.1 to 0.13 mm. In total preparations (Fig. 1) it is hardly to be seen, since it rises directly toward the observer. Its walls are thin and terminate at the genital cloaca in a short projecting muscular papilla (Fig. 4). This papilla, which is $55\ \mu$ long and $25\ \mu$ in diameter, is the male copulatory organ, and may be regarded as the morphological equivalent of the cirrus,* although differing much from the usual form of that organ, this *Distoma* being thus intermediate between those with no cirrus and those in which a powerful muscular organ is present. The section figured is oblique and passes out of the lumen into the wall of the organ at its proximal portion.

First of the female sexual organs is the germarium, an almost spherical mass of deeply staining polygonal cells lying to the right of the acetabulum, which it far surpasses in size. The contour of the mass is very perfect and I have been able to detect the germiduct only on sections. It is a narrow, delicate walled tube leading, often with one or two bends, to the center of the shell gland, which lies near the dorsal surface just behind the acetabulum. This gland consists of a mass of flask-shaped cells, the rounded ends of which are at the periphery of the gland, whereas their necks (ducts) abut upon the ootype or the continuation of the germiduct through the center of the shell gland. The relations here will be clearer after describing the vitellaria or yolk glands and their ducts.

The vitellaria are easily recognized by their brown color, though they vary in size and slightly in position with the age of the specimen. In the youngest each consists of five to eight approximately spherical lobes near the posterior end of the worm, one such gland being found on each side. As the *Distoma* becomes more mature these glands increase in size until they reach anteriorly to the testes, overlapping the latter on their lateral edges and extending almost as far as the limit of the ventral sucker. In older specimens I am

* Braun's criticism that different parts are confused under this name is evidently true.

inclined to think that they are again reduced in size, but the evidence on this point is incomplete. The vitelline ducts appear first on the anterior edges of the glands, extend anteriad or merely transversely, according to the development of the vitellaria, to the level of the center of the shell gland. Since in most specimens they are filled with brown yolk matter, they can be easily followed; they pass into the shell gland from below, unite below its center to form a small median yolk reservoir, from which a short duct rises directly dorsad to join the ootype at the point where the cells of the shell gland terminate.

From the edge of the shell gland the female sexual duct becomes the uterus and proceeds abruptly to the ventral surface, near which it begins its winding course. Its coils fill the greater part of the body between the organs of the posterior half (Fig. 3), and finally, near the posterior end, it passes abruptly into the metraterm,* a muscular tube which leads directly forward to the female genital pore. The walls of this tube are nearly in contact, and one finds but occasional eggs within it.

The eggs measured from the living specimen averaged 34μ by 17μ , and from the alcoholic material 30μ by 14μ . In one specimen of the latter they reached 45μ by 20μ , which is the extreme size found. They are elliptical, with a light-brown shell, having a cover (Fig. 5).

One organ has thus far been left out of account. Just at the posterior edge of the shell gland and a little to the left of the median line a narrow canal leads in from a minute opening on the dorsal surface. It is the so-called Laurer's canal, and shows here a lumen of 2μ to 3μ in diameter, with a homogeneous wall of about the same thickness, around which are, in a layer, cells with deeply staining nuclei. This canal passes first ventrad, then toward the center of the shell gland, and there joins the ootype about where the vitelline duct connects with it. Owing to the extreme minuteness of the

* Braun (93) has well put the objections to the use of the term "vagina" for this portion of the uterus. I should like to propose the designation *metraterm* (*μήτρα*, the uterus, and *τέρμα*, end) for this region, which he calls "Scheidentheil." Much confusion has arisen in other groups from the use of the same name for parts differing morphologically, and it seems unwise to employ here a term which applies to another organ in the nearly related Monogenea and Cestoidea, especially since the homology of Laurer's canal to the vagina of those groups is at present so generally accepted. The designation *metraterm* would apply equally well to the end of this uterus, whether used in copulation or not.

parts concerned, this terminal portion was not fixed with absolute certainty, since both were not found in the same series of sections. I was unable to find any seminal receptacle and believe that none exists in this species.

During the time in which the Distomes of the large catch already mentioned were kept in a small dish of salt solution copulation took place in a number of cases. It was possible to preserve the pairs in corrosive sublimate without their becoming detached and to observe the anatomical features on sections later. This is the only case of which I can find record in which any observations have been made on the copulation of a *Distoma* without a well-developed cirrus. The organs are too small and the living specimens too opaque that more could be determined from them than the relative position and attachment of the two individuals. They lay with the ventral surfaces opposed so firmly united that it was impossible to force them apart by any pressure on the cover-glass. Like the case of *D. clavigerum* recorded by von Linstow (90), the longitudinal axes formed an angle with each other, yet here so small that the oral suckers lay but a very short distance apart and unconnected. The examination of sections showed that the two were so firmly joined by the ventral suckers that the body wall of one was drawn up into the acetabulum of the other in the shape of a large knob. The sucker was contracted, so as to be deeper than wide, and its cavity was entirely filled with the projection from the body wall of the other. The same was true of the other individual. So firm was the hold of the one on the tissue of the other that the knob broke off transversely rather than withdraw from the sucker of the other. The cloacæ of both were everted so that there remained but a shallow depression with a low ridge to mark its margin. I am unable to say that the ridges of the two were exactly in contact, though they lay near each other in the sections. The short conical papilla already described was somewhat elongated and widened almost to a cylinder, with a length of 60–75 μ , a wall 5 μ in thickness, and a lumen of 7.5 μ , through which a stream of spermatozoa was passing out from the vesicula seminalis. The papilla was inserted in the metraterm, and the spermatozoa already introduced could be traced as far as the transition from this tube to the egg-filled uterus. The copulation was mutual, and the cirrus papillæ of the two when viewed on frontal sections cross at a small angle, each pointing slightly toward the anterior end of the other. This is due

to the very convex form of the acetabulum in this act, since it forces the metraterm to make a slight bend toward the anterior end before passing above the sucker and posteriad.

The animals found in copula were without exception adults, and had the uterus more or less filled with developing eggs, so that fertilization must have taken place ere this. It is probable that the act is performed several times in the lifetime of an individual, and, on the other hand, it seems clear that self-fertilization must also take place, since in those removed from the cysts in the crayfish I found in every case a larger or smaller mass of eggs in the uterus. In each cyst is enclosed but a single individual, so that cross-fertilization seems to be entirely excluded.

There is strong evidence that, in this species at least, Laurer's canal cannot be functionally a vagina. Its size is such as to preclude apparently the possibility of introducing even such a small cirrus papilla as exists here, and I have been able to find nothing but an occasional yolk particle in it. Furthermore, while the large number of *Distomes* was in the small dish of salt solution, in active motion and often in copula, as already described, no instance was found in which one specimen was attached even lightly for a few seconds to the dorsal surface of another, though they were continually moving over each other in their wanderings. In short, all the evidence which could be found militated against the use of the canal for such a purpose. This question is fully discussed in Braun (93, p. 752), where attention is called to the lack of positive knowledge on the subject. I believe that this case furnishes a negative argument which can be overthrown only by positive evidence to the use of the canal as a vagina.

Life History.

The encysted *Distoma* was found in *Cambarus propinquus* Ger. and in each specimen numerous cysts. The cysts occupied the space in the cephalothorax above the heart and sexual organs. On the average, a cyst measures 1.28 by 0.9 mm. and presents a characteristic appearance (Fig. 6). The alcoholic specimen has the form of a regular ellipse, near the center of which is a U-shaped area of a clear amber color; it is the excretory reservoir with its two anterior horns. At either side of this lies a mass composed of three to five lobes each. These are colored dark greenish brown and represent the lobed vitelline glands. The line which crosses the ellipse longitudinally

is the posterior limit of the body of the *Distoma*. Specimens shelled out from these cysts and mounted after staining show the same structure in detail, so that the connection of the two can hardly be doubted.

According to Forbes (80) the crayfish forms part of the food of the following lake fish: *Ameiurus natalis* (Le S.), *Ambloplites rupestris* (Raf.), *Micropterus dolomieu* Lacép., *Ictalurus punctatus* (Raf.), and *Perca flavescens* (Mitch.). The first two were not infected, nor was the third, although, according to Forbes, 62 per cent. of its food is made up of crayfish. In those examined at New Baltimore no trace either of a crayfish or of this parasite was discovered in its alimentary canal. Both *Ictalurus* and *Perca* contained specimens of this species, though not in the same mass as the dogfish.

Other *Distomata* have been found in the crayfish in this country in the encysted stage. Kellicott (83) was one of the first to call attention to this fact in the Proceedings of this Society. Wright (84) gives an account of a form, probably *D. nodulosum* Zed., from *Cambarus propinquus*. Linton (92) refers to the discovery of the same species in the "common" crayfish in Michigan. I have frequently seen this form at Ann Arbor, and the adult was discovered in the work of the past summer, but it will be considered in another place. There is no possibility of confusing these with the species described above on account of the considerable difference in the size, shape, and appearance of the cysts. Our *Distoma* is equally distinct from *D. isostomum* Rud. and *D. cirrigerum* v. Baer, the species which infest the European crayfish.

Of all species of the genus *Distoma* to which I have been able to refer, this one most resembles *D. pygmaeum* Lev.,* from the eiderduck (*Somateria mollissima* Leach). The difference in size is very great, and there are slight differences in the location and proportions ~~and~~ of organs and in the size of the ova.

Specific Diagnosis.

Distoma (Brachycelium) opacum, H. B. Ward, 1894.

Length, 1.6 to 1.7 mm.; breadth, 1 to 1.16 mm. Living specimen pyriform to gourd-shaped, white with yellow-brown posterior portion. Oral sucker subterminal, 0.13 to 0.16 mm. in diameter; acetabulum larger, sessile, near center of ventral surface, 0.17 to 0.21 mm. in diameter.

* I am indebted to my friend Dr. C. W. Stiles, of the Bureau of Animal Industry, for a reference to this form and for a sketch of it.

Pharynx small, slightly removed from oral sucker; œsophagus long, crura intestini short, clavate. Testes two, postacetabular, lateral, anterior to the few-lobed vitellaria. Cirrus rudimentary. Seminal vesicle to the left of acetabulum near median line, about the same size as the ovary at the right of the ventral sucker. Genital pore immediately adjacent to acetabulum on the left. Ova elliptical, $30\ \mu$ to $40\ \mu$ by $17\ \mu$ to $20\ \mu$, with cover.

Hosts: *Amia calva* L., *Ictalurus punctatus* (Raf.), *Perca flavescens* (Mitch.), in Lake St. Clair. Encysted in *Cambarus propinquus* Ger., ibidem.

Type specimens: In collections of Michigan Fish Commission and of H. B. W.

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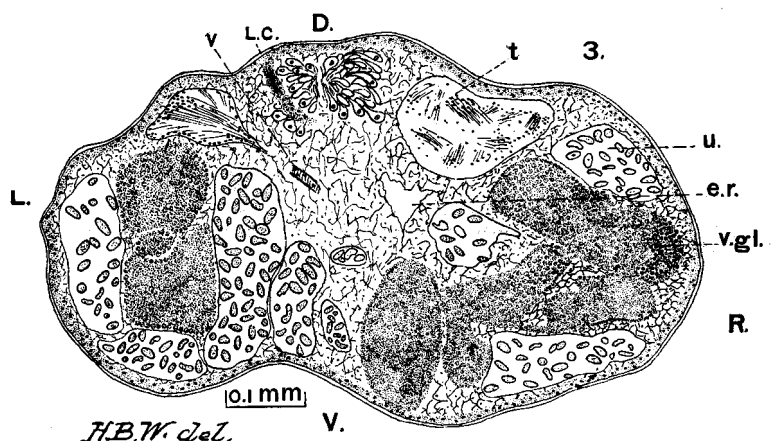
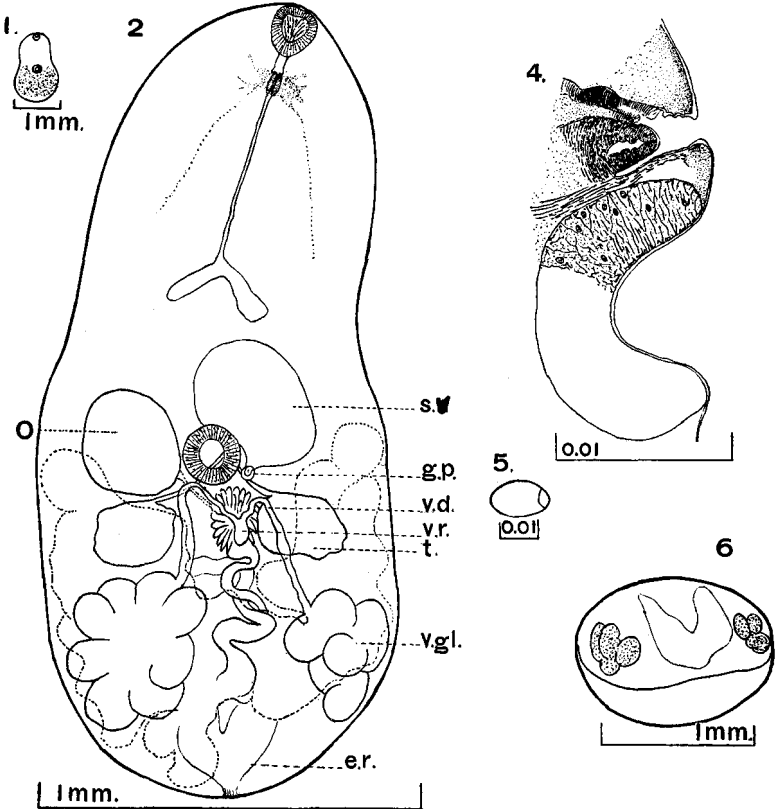
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Explanation of Figures.

- Fig. 1.—Ventral view of alcoholic specimen, drawn under an embryo-graph. $\times 9$.
- Fig. 2.—Ventral view of a specimen preserved in corrosive sublimate, stained in Czokor's alum cochineal, and mounted in balsam. O = germarium; s. v. = seminal ~~receptacle~~ ^{vesicle}; g. p. = genital pore; v. d. = vitelline duct; v. r. = vitelline reservoir; v. gl. = vitelline gland; t. = testis; e. r. = excretory reservoir. The uterus was omitted to avoid confusing the figure. The dotted line represents the limit of its coils filled with eggs. The germiduct, represented by a double dotted line from the germarium to the shell gland, was supplied from sections. Abbe camera. Zeiss AA 1. $\times 75$.
- Fig. 3.—Transverse section from a specimen preserved in corrosive sublimate, stained in Ehrlich's acid hæmatoxylin, and mounted in balsam. L. c. = Laurer's canal in the edge of the shell gland; t. = testis; v. gl. = vitelline gland; e. r. = the extreme anterior tip of one horn of the excretory reservoir; u. = coils of the uterus; v. = metraterm; D = dorsal; V = ventral; R = right; L = left. Camera outlines. Zeiss AA 3. $\times 125$.
- Fig. 4.—Transverse section of the ventral sucker, genital cloaca, and sexual pores. The opening of the male duct is in the small muscular papilla next the sucker, that of the metraterm being further laterad. Specimens preserved in Flemming's solution sections mounted unstained. Abbe camera. Zeiss C. 3. $\times 340$. The scale was cut away from this figure by the engraver.
- Fig. 5.—An egg from an alcoholic specimen. Abbe camera. Zeiss C. 4. $\times 400$. The length of the measuring line in this figure should be printed 0.07 mm.
- Fig. 6.—Cyst from *Cambarus propinquus*, drawn in alcohol. Abbe camera. Zeiss AA 1. $\times 30$.

The magnifications in the explanation of figures refer to the original drawings, which have been reduced to about two-thirds in the process of reproduction.



H.B.W. del.