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### 3.—ON TWO SPECIES OF LARVAL DIBOTHRIA FROM THE YELLOWSTONE NATIONAL PARK.

BY EDWIN LINTON.

[Plates XXIII to XXVII.]

In December, 1889, I received an interesting lot of entozoa, collected in the Yellowstone National Park by Dr. David S. Jordan of Bloomington, Ind., chiefly during October, 1889. The collection submitted to me for examination consists of two trout (*Salmo mykiss*), with the viscera of three others, four suckers (*Catostomus ardens* J. & G.), and a few large *Ligulæ* that had been removed from the abdominal cavity of the latter host. The trout were obtained in the Yellowstone River just below the lake, while the suckers were from Witch Creek, a hot tributary of Heart Lake.

Dr. Jordan states that the parasites of the trout first appeared, so far as he observed, in cysts among the pyloric cœca, later in the liver and among the viscera, and finally reaching a length of 5 inches in the flesh of the abdomen. These parasites were found in all trout in lakes fed in part by geysers, the trout abounding in the warm water in consequence of the abundance of food there.

Of the suckers he states that they abound in the warm waters, ascending to the temperature of 80° or more. About one in four has a very large parasitic worm in the abdominal cavity, where it is often as large as the whole viscera, and lies along the middle line of the belly. These worms were often more than a foot long and  $\frac{1}{4}$  inch broad.

#### REMARKS ON THE GENUS LIGULA.

The generic name *Ligula* has long been used for certain cestods of the family *Dibothriidae* (*Pseudophyllidae* Van Beneden). The genus was distinguished from the genus *Dibothrium* (*Bothriocephalus*) chiefly by the absence of distinct segments in the body. The forms referred to this genus are common in many of the fresh-water fishes and are especially abundant in the *Percidae* and *Cyprinidae*, where they occur in the abdominal cavity and body-wall of their host. The adult stage has not been found in fishes.

The admirable researches of Duchamp (1876) first proved the identity of the *Ligulæ* of fishes with forms which are adult in the intestines of different aquatic birds. Duchamp's investigations were on an abdominal *Ligula* of the tench (*Tinca vulgaris*). This *Ligula* has a most extensive synonymy, but the name which has priority is *L. simplicissima* Rudolphi. Diesing (1864) recognizes two species of *Ligula*, viz, *L. monogramma* Creplin (a synonym of *L. simplicissima*) and *L. digramma* Creplin.

It is proposed by Donnadieu (1877) to unite all the species of *Ligulæ* in one and the same species, in the genus *Dibothrium* with the specific name *Dibothrium ligula*.

I agree with Dr. F. Zschokke (*Recherches sur l'Organisation et la Distribution Zoologique des vers Parasites des Poissons D'Eau Douce*, 1834), that the generic characters of *Ligula* are identical with those of *Dibothrium*. With regard to Donnadieu's proposed union of the different species, to which proposition Zschokke assents, I am not prepared to express an opinion, my investigations having been limited to the forms which furnish the subject matter of this paper. While the species which I have for convenience named *L. catostomi* appears to be identical with *L. simplicissima*, the other, *Dibothrium cordiceps* Leidy, presents some striking differences.

Although these entozoa are in all probability larvæ of *Dibothria*, the name *Ligula* has been in use so long that I deem it best for the present to retain it for the sucker parasite as a designation of this particular form of *Dibothrium* larva.

*Ligula catostomi.*

[Pl. XXIII, Figs. 1-5; Pl. XXIV, Figs. 1-6; Pl. XXV, Fig. 1.]

(*Dibothrium ligula* Donnadieu?)

*Ligula simplicissima* Rudolphi.

*Ligula monogramma* Creplin.

Upon opening the body of one of the infested suckers the cavity is found to be almost completely filled with the contained parasite or parasites (plate XXIII, fig. 1). The presence of the parasite is indicated before the body is opened by the somewhat swollen condition of the abdomen. In some cases the abdominal cavity was found to be so packed with these worms that the heart, stomach, liver, and spleen were crowded into a very small space in the antero-dorsal part of the cavity, while the intestine was intertwined with the body or bodies of the parasites.

The parasites are not covered by any special cyst or membrane, but lie free in the body cavity. Usually but a single worm occurs in the host, but in one instance three were found in the abdominal cavity of a single sucker. The specimens, while presenting some differences in size, agree in their general form and outline, as well as in the details of their very simple superficial structure. They all evidently belong to the same species. On account of the extreme simplicity of structure, however, it is very difficult to determine their exact specific relation to European forms. Since the *Ligulae* attain their sexually mature state in the intestines of certain piscivorous birds it follows that this species should have a wide geographical distribution.

The sketches which accompany these notes show sufficiently well the external character of this parasite. In general outline they are strap-shaped, attaining their greatest breadth a short distance back of the anterior end, from which point they taper slightly towards the anterior end, and gradually towards the posterior end. The anterior end is usually broad and bluntly rounded; in one case, however, it was observed to be slightly appressed into a short, blunt, subcylindrical termination (plate XXIII, fig. 5). At the extreme anterior tip there is usually a median longitudinal sulcus extending back a short distance on each lateral face. This appears, in section, to be the beginning of the adult bothrial depressions (plate XXIV, fig. 1). From about the anterior third or a little forward of that point, the body tapers slowly but rather uniformly to the posterior end, which terminates at last somewhat abruptly in a rather sharp point. The surface of the body appears to the naked eye to be smooth or nearly so, but with the aid of a simple lens it is seen to be crossed by fine transverse grooves and wrinkles. The grooves or striæ are shallow and do not divide the body into segments. They

may be regarded, however, as the first indications of the strobile condition, in which the segments are but little individualized. Usually there are a few lateral longitudinal striæ, and invariably a rather strongly marked median furrow, which begins a short distance behind the anterior end and extends almost to the posterior end, becoming more evident in the median and postero-median regions of the body. When magnified a few diameters this median line appears to be made up of a row of punctate depressions, very near together, but approximating a zigzag line, indicating what is shown more plainly, in sections, that the reproductive openings are arranged, in the adult, along the median lateral line.

The largest specimen measured 28.5 centimeters in length. At the anterior end it was 8 millimeters broad; 7 millimeters back of the anterior end the breadth was 11 millimeters; near the anterior end the breadth was 1.5 millimeters. The thickness throughout was about 2 millimeters.

Another specimen had the following dimensions: Length, 19.3 centimeters; diameter of compressed head, 2.5 millimeters; breadth near anterior end, 6 millimeters; greatest breadth, 70 millimeters from the anterior end, 9.5 millimeters; breadth near posterior end, 2 millimeters, tapering thence to a point; thickness about 2 millimeters.

Three specimens from a single host were 120, 110, and 106 millimeters in length, respectively. The breadth near the anterior end of each was 4 millimeters; the greatest breadth of No. 1 and No. 2 was 8 millimeters; of No. 3, 6 millimeters; the breadth of each near the posterior end was 1.5 millimeters. In No. 1 the greatest breadth was about 27 millimeters from the anterior end; in Nos. 2 and 3 it was about 15 millimeters from the anterior end.

The specimen mentioned above which measured 19.5 centimeters in length came from a fish which measured 11 centimeters in length, exclusive of the tail-fin. Since it was not possible to straighten the worm without breaking it, and since the alcohol had doubtless caused it to contract more or less, the length obtained by measuring the alcoholic specimen is certainly less than that of the living specimen. The weight of the host was in this instance 20.7 grams; the weight of the parasite was 2.65 grams, or about 12 $\frac{3}{4}$  per cent. of that of the host.

In a small sucker, 9.5 centimeters in length, exclusive of the caudal fin, three parasites were found, the measurements of which are given above. The weight of the host was 9.1 grams, while the combined weight of the three parasites was 2.5 grams, or nearly 27 $\frac{1}{2}$  per cent. of the weight of the host. Or, to make a parallel case, in order to infest a man weighing 180 pounds to an equal degree it would require nearly 50 pounds of tape worm.

Among the specimens which had been separated from their respective hosts before coming into my possession I find one that is quite different in shape from the others. It is 40.5 millimeters long and 10.5 millimeters broad and terminates bluntly at each end. It is probably the anterior end of a large specimen which has been broken while living so as to allow the muscles to contract strongly and obscure the broken part.

*Anatomy.*—On account of the few external characters of which use can be made in the identification of these specimens, it became necessary to make some investigation into the histological structure. In these investigations portions were stained with harmatoxylon, borax carmine, Bismark brown, etc. The most uniformly satisfactory results were obtained with borax carmine, Grenacher's formula, 35 per cent. alcohol.

The specimens had been lying in alcohol about three months. They had not been especially prepared for histological study, and it is probable, therefore, that many interesting features in the finer anatomy have not been brought out in my researches. All the descriptions of histological structure in this paper, as well as the sketches which are appended, are based on the carmine preparations.

*Musculature and body-layers.*—The layers of the anterior region of the body, where they are unmodified by the incipient genitalia, have the following arrangement: There is first a thin outer cuticular layer. This is structureless, but appears to be continuous within, with a series of longitudinal muscles. The latter, in transverse sections, present the appearance of radial plates, attached to the outer cuticular layer (plate XXIV, fig. 6). The interstices between the plates of longitudinal muscles are filled with a granular or nuclear protoplasm which is strongly stained. Towards the inner portion of this granulo-fibrillar layer there are numerous calcareous bodies. Next within the granulo-fibrillar layer is a porous or vascular layer, in which a few fine connective fibers and protoplasm, with abundant nuclei, can be distinguished. The loose, open character of this layer is due to the numerous peripheral vessels of the vascular system (plate XXIV, fig. 6, *f*). Next is a thick layer of strong, longitudinal muscles. In transverse sections the bundles of fibers of this layer are seen to be separated by plates of radial fibers, which cross from one lateral face of the body to the other, being reduced to very fine fibers in the peripheral regions, except in the subcuticular layer, where the plates of longitudinal muscles are probably derived from them. In longitudinal sections, parallel to the lateral faces, the radial plates appear as short connecting bands between the bundles of longitudinal fibers (plate XXIV, fig. 5). There are very few calcareous bodies and little or no granular protoplasm in the layer of longitudinal muscles. Next is a layer of coarse, strong, circular muscles or, more properly speaking, fibers running transversely from margin to margin, and surrounding a central space, which represents, in transverse sections, a central core of the body. The layer of circular fibers is crossed, like the longitudinal layer, by radial fibers, which in the inner parts of the layer are distinct, but in the outer portion begin to be collected into bundles, which, in turn, become the radial plates of the longitudinal layer. In the outer part of the circular layer, where the radial fibers are collecting into bundles, transverse sections show a reticulated structure, made by the crossing of the radial bundles and the circular fibers, which here also form bundles. In the meshes of the net-work thus formed are a few longitudinal fibers. Calcareous bodies are sparsely scattered through this layer, while nuclei are somewhat abundant. The inner core is crossed by numerous parallel fibers, running from side to side of the body, and which are continuous with the radial fibers of the circular and longitudinal layers. In the anterior part of the body these fibers predominate, but in the median and postero-median regions of the body they become much attenuated and scattered. There are also a few fine fibers transverse to these, *i. e.*, running from margin to margin. The inner core, in the anterior part of the body, is thickly beset with calcareous bodies, and contains very numerous nuclei.

In the above description regard is had mainly to the arrangement of layers as they occur in the anterior regions of the body. In sections made from portions taken from near the posterior end the different layers will be found to have undergone much modification, although the general distinctive character of each remains. The radial fibers, or, better, those which are parallel with the smaller diameter of the elliptical cross-

sections, suffer most change, being reduced to slender filaments. Their substance in the inner core is plainly lost to the genitalia which are already taking shape.

*Vascular system.*—The vascular system is represented by two sets of vessels which may, for convenience, be designated (1) peripheral, and (2) central or centro-marginal.

The peripheral vessels constitute a net-work of vessels which lies in what was called above the porous or vascular layer, between the subcuticular granular layer and the inner longitudinal layer. In this set of vessels there are a great many longitudinal vessels with numerous anastomosing branches. In both longitudinal and transverse sections many branches were seen to leave the peripheral layer, and penetrating the longitudinal muscle layer debouched into one of the longitudinal central vessels of the inner core. Near the anterior end of the body the peripheral vessels are most numerous, forming there an intricate net-work. Towards the middle of the body they become reduced in number, and assume more the character of longitudinal vessels. This character is retained near the posterior end, where the number of these vessels shown in transverse sections along each lateral border of a section is five or six. In some of the first sections of the anterior end the peripheral vessels were seen spread over the central part of the sections, showing that the vascular layer closes in the anterior end of the body along with the cuticular and subcuticular layers (plate XXIV, fig. 1). Their disposition at the extreme posterior end was not made out.

The second set of vessels are the usual centro-marginal vessels peculiar to the cestods. Of these there are two sorts, corresponding to what may be seen in adult *Dibothria*. In brief, these two sorts may be characterized as follows: The first sort consists of two conspicuous vessels, one lying towards each margin, without proper walls, excepting a few circular fibers and numerous nuclei, the lumen being filled with spongy tissue, which does not stain strongly with carmine, but contains a few nuclei, similar to those which surround the vessels (plate XXIV, fig. 2, *a d*). The second sort consists of a varying number of vessels more numerous towards the anterior end, with proper walls and an open lumen (plate XXIV, fig. 2, *b*).

I shall, for convenience, speak of the former as the marginal canals, and the latter as the aquiferous vessels.

The *marginal canals* make their appearance in sections, made very near the anterior end, as two nearly circular spots of spongy or areolar unstained or slightly stained tissue near the center of the sections. A line joining their centers corresponds with a line drawn from margin to margin. They lie directly between the lateral notches which mark the rudimentary bothria. At first they almost coincide, so that they appear to communicate. They soon separate, however, as they are traced posteriorly, and at a distance of 2 or 3 millimeters from the anterior end they are situated at about the same distance from the margins as they are from each other. Near the middle of the body they are relatively nearer the margins, the distance from one canal to the other being about one and two-thirds times the distance from a canal to the nearest margin. Towards the posterior end they approach relatively nearer the margins. These canals preserve their distinctive character throughout their whole extent. They do not in any way resemble the aquiferous vessels. Instead of being hollow tubes, as is the case with the latter, they are filled with a spongy connective tissue, which is very slightly affected by carmine staining. They are nearly circular in outline, and are limited by a few circular fibers in which there are numerous nuclei, in some of

which a nucleolus was differentiated by the staining fluid. Nuclei of the same nature, especially near the anterior end, were also seen sparsely scattered through the spongy tissue. In a few cases some irregular patches of granular protoplasm were observable among the spongy connective tissue. No branches were certainly made out leading either to or from the marginal canals. The marginal canals are throughout much larger than the aquiferous vessels. Their diameter is from .06<sup>mm</sup> to .08<sup>mm</sup> in the anterior and median regions of the body; towards the posterior end, however, they become somewhat smaller.

These canals are evidently the *lateral canals* of Duchamp, and the *plasmatic vessels* of Kuchenmeister and Zurn.

The *aquiferous vessels* in transverse sections of the anterior portion of the body appear to be of variable number owing to the fact that branches from the peripheral vessels join them at frequent intervals. The branches usually unite with the central vessels at an acute angle so that the cut ends of the branches cannot be distinguished from the cut ends of the main central vessels.

Near the anterior end there are eight principal vessels lying in two groups of four each, central to what I have designated the marginal canals. This disposition is not invariable, for in many sections more than four aquiferous vessels may be seen in the vicinity of one of the marginal canals.

Cross-sections of these vessels made near the anterior end of the body were oval in outline with the longer diameters varying from .014 to .03 millimeters and the shorter diameters from .01 to .014 millimeters. In both longitudinal and transverse sections the appearance is that of a hollow tube with a definite wall differentiated from the surrounding parenchyma and fibrous tissue. In cross-sections, the walls appear to be structureless. The fine longitudinal and circular contractile fibers seen in the walls of the aquiferous vessels in *D. cordiceps* were not observed in any of the longitudinal sections of *L. catostomi*. The thickness of the walls is about .0025 millimeters. Reference to the measurements which I have given and to the sketches will show that the aquiferous vessels are throughout much smaller than the marginal canals. The difference between the two is perhaps best shown in longitudinal sections, where the walls of the aquiferous vessels are seen to be more or less folded, but everywhere distinct from the surrounding tissue, and the lumen free from tissue of any sort. A longitudinal section of a marginal canal, however, shows a slightly sinuous canal filled with a fine fibrous tissue, appearing irregularly striated. Both sorts of vessels are surrounded by numerous nuclei, which in the case of the canals are in part entangled in the circular fibers which limit the canals, while in the case of the aquiferous vessels they do not enter into the structure of the vessel walls.

In the median and posterior parts of the body where the genitalia have already begun to develop the aquiferous vessels appear to be reduced to two, one at a short distance from and central to each marginal canal.

The aquiferous vessels in the median region of the body were about .013 millimeters in the smaller and .019 millimeters in the greater diameter, outside measurement, and .008 millimeters by .013 millimeters inside measurement. In this region the walls of the tubes are quite sharply defined and in some cases even slightly separated from the adjoining tissue.

I have not been able to make out any communication between either the periph-

eral or the central aquiferous vessels on the one hand, and the marginal canals on the other.

*Calcareous bodies.*—These are numerous, especially in the anterior regions of the body. They are confined for the most part to the central core and to the peripheral region. In the latter they are nearly all found between the cuticle and the peripheral vascular layer. In the median regions of the body they have almost disappeared from all parts except the peripheral layer and there they are not abundant. In sections from the posterior parts of the body, where the reproductive organs have begun to develop they are yet more sparsely scattered and are confined almost exclusively to the peripheral region. In size these calcareous particles vary. The larger ones measure about .008 by .01 millimeters in the two diameters. Most of them are circular or oval in outline, but some are irregular. A great many of them show a concentric structure in optical section.

*Reproductive organs.*—In these ligulæ from the abdominal cavity of the sucker the reproductive organs have reached a comparatively advanced stage of development.

Both longitudinal and transverse sections from the middle and posterior parts of the body show clusters of nuclei which are deeply stained by carmine. These clusters lie in the inner layer of longitudinal muscle fibers, near one of the lateral faces. In longitudinal sections made parallel to the lateral faces of the body they are seen to be arranged along the median line in a somewhat zigzag row, and correspond in position to the genital openings which are also revealed in those sections which are carried through the superficial layers of one of the lateral faces. In transverse sections each of these clusters of nuclei is seen to consist of two clusters lying side by side (plate XXV, fig. 1). They are the rudimentary genital organs. The one will, in the adult, give rise to the cirrus and its pouch, and the spermatheca; the other to the vagina, uterus, ovary, etc. In the peripheral region the vitellaria are plainly indicated by a nuclear layer, which is separated from the cuticle by a thin layer of longitudinal fibers and extends to the inner layer of longitudinal muscles. The nuclei which lie in the interstices of the external layer of longitudinal muscles and those which are so abundant in the vascular layer in the anterior part of the body evidently contribute to the formation of the vitellaria.

The beginning testes are plainly indicated in transverse sections of the postero-median and posterior regions of the body. These consist, in each section, of a series of a dozen or more nests of nuclei lying towards each margin, extending from near the margin towards the center a distance equal to about one-third the diameter from margin to margin (plate XXIV, fig. 3d). These lie in the central core. One of these nests of nuclei is shown in section, highly magnified in fig. 42, plate XXIV.

Nuclei, which in the anterior regions of the body are more or less abundantly disseminated among the muscular and fibrous layers, are, in the median and posterior regions, confined to the peripheral and central regions, where they have already begun to collect to form the genitalia. The transverse fibers which in the anterior regions are abundant and strong, especially in the central core, and circular layer, are in large measure reduced to very fine fibers in the median and posterior regions. The various tissues of the body have already in great degree been absorbed to contribute to the formation of the genitalia. The inner layer of longitudinal muscles appears to have suffered the least from absorption thus far.

## LIGULA CATOSTOMI FROM STOMACH OF A TROUT.

On February 8, 1890, I received from Professor Jordan some fragments of ligulæ from the stomach of a trout (*Salmo mykiss*).

These specimens were collected July 20, 1889, at Twin Lake, Colorado.

These fragments are in six or seven pieces, but are plainly pieces of the same worm. Their combined length is about 18 centimeters, greatest breadth 1 centimeter, and maximum thickness  $3\frac{1}{2}$  millimeters. The ends of the fragments present a frayed appearance, the surface is broken, pitted, the cuticle removed in places, and a general look of incipient disintegration, from which I infer that the specimen has been taken into the stomach of the trout along with its proper intermediate host. It is without doubt identical with *Ligula catostomi*. The shape, transverse striæ, and longitudinal furrows, with the profound median lateral furrow, all point conclusively to this identification. Neither the anterior nor the posterior end remains intact, and I find from superficial examination no evidence of any further approach to the adult condition than maintains in the specimens from the abdominal cavity of the sucker.

There can be but little doubt therefore that *Salmo mykiss* is not the proper host of the adult stage of this worm.

Further examination of the entozoa of the fish, and also of the piscivorous birds of the Yellowstone region will doubtless yield the necessary material for completing the history of this parasite.

*Dibothrium cordiceps* Leidy.\*

[Pl. XXV, Figs. 2-5, Pl. XXVI, Figs. 1-5, Pl. XXVII, Fig. 5.]

These ligulæ of the trout (*Salmo mykiss*) were found, not free in the abdominal cavity, as was the case in those from the sucker, but were inclosed in the muscular walls of the abdomen. The specimens were collected in the Yellowstone River, just below the lake, October 10, 1889.

Smaller forms, apparently of the same species, were found encysted among the pyloric cæca, in the liver and in the serous covering of the stomach and intestine. These are described below. The specimens from the abdominal walls were in cavities lined with connective tissue, the cavities being from 1 to 3 centimeters in diameter.

Two trout sent by Dr. Jordan were examined. Each of these had a worm inclosed in the muscular walls of the abdomen. In one the worm was situated about  $2\frac{1}{2}$  centimeters in front of the ventral fin. It lay amid the muscles, but had displaced those lying immediately above and below, so that it lay contiguous to the peritoneum and was separated from the skin by a thin layer of fatty tissue. In a piece of the abdominal wall of another trout, the ligula lay in an elongated and somewhat irregular cavity tunneled out of the tissues, in all some 3 centimeters in length. The cavity in both cases was lined with connective tissue (plate XXV, fig. 5).

These specimens are very much crumpled and folded on account of having been hardened in alcohol while still confined in the narrow limits of their cysts. It is therefore difficult to obtain good measurements of them. One of the longest was measured after it had been straightened out as well as could be done in its crumpled condition. It was 15 centimeters in length. The diameter of the cylindrical anterior end was about 1 millimeter near the end, whence it tapers to a blunt point. The greatest

\* Hayden's report of U. S. Geological Survey for 1871, pp. 301-2.



breadth was 3 millimeters; the thickness about 1.25 millimeters. The posterior extremity of this specimen was truncate and slightly emarginate, and about 2 millimeters broad. Anteriorly the body is somewhat tapering, rather cylindrical, and extremely irregular in outline. The body is crossed by fine transverse striæ which appear to be incipient joints. Towards the posterior and larger end these striæ become more distinct and therefore the body there assumes a decidedly segmented appearance.

*Anatomy.*—The following remarks on the anatomy and the sketches are based on sections of portions stained in toto in borax carmine.

*Musculature.*—The musculature is very similar to that of *L. catostomi*.

The layers of the body from without inward are, first a cuticular layer; next a layer of longitudinal and radial fibers with much granulo-nuclear protoplasm interspersed; next a vascular layer in which the peripheral system of aquiferous vessels is well represented along with many nuclei and, in the antero-median and median parts of the body, numerous calcareous bodies. This layer merges into a granulo-nuclear layer within, and is succeeded next by a prominent layer of longitudinal muscles. The latter is separated from the inner core by a narrow layer of circular or transverse muscles. No definite system of muscle fibers was made out in the inner core in median section; anteriorly the arrangement is like that of *L. catostomi*. The parenchyma there shows an irregular net-work of connective fibers, with numerous nuclei and immense numbers of calcareous bodies interspersed. In the near vicinity of the marginal canals and around the aquiferous vessels nuclei are very abundant. Sections of the anterior end show the presence of the two opposite bothrial pits, characteristic of the genus *Dibothrium* (plate XXV, fig. 2, e).

*Vascular system.*—This, with the exception of a feature to be mentioned presently, appears to be much like that of *L. catostomi*. In sections of the anterior end several small vessels were observed in the peripheral region. These became even more prominent in sections farther back.

The *marginal canals* soon make their appearance. As in *L. catostomi*, they are larger than the central aquiferous vessels, do not have a distinct wall, and the lumen, instead of being open, is filled with pale, unstained, spongy connective tissue, appearing somewhat fibrous in longitudinal sections. They are densely surrounded by nuclei. Near the anterior end the transverse section of one was .05 by .03 millimeter in its two diameters. A few sections of the head were characterized by a distinct line of nuclei connecting the two marginal canals (plate XXV, fig. 2, d). The marginal canals retain their distinctive character to the posterior end. In longitudinal sections they are seen to pursue a gently undulating course.

The *aquiferous vessels* of the inner core appear in sections near the anterior end as two principal vessels which lie near the central border of the marginal canals. As in *L. catostomi*, these vessels have a distinct wall and an open lumen. One of these vessels, in the same section from which the diameters of the marginal canal given above were obtained, measured .019 millimeter in its outer and .011 millimeter in its inner diameter. The walls of the aquiferous vessels are quite distinct from the surrounding tissue and present on the outer surface, in cross-sections, a peculiar roughened appearance, as if thickly beset with minute bristles. The inner surface of the wall appears smooth in cross-sections. In longitudinal sections made in the postero-median regions of the body the aquiferous vessels were seen to pursue a somewhat tortuous course, and their folded and erumpled walls cut through looked like a series of frills or ruffles.

Fine longitudinal and circular contractile fibers were distinctly visible. (plate XXVII, fig. 5.)

Longitudinal sections of the posterior end reveal a number of rudely circular open spaces lying in the vascular layer. Some of them are lined with a layer of fibrous tissue in which are numerous nuclei. Others are without any proper lining. The sections were made parallel with the lateral faces of the specimen. Approaching from one end of the series the sections contain from two to four of these cavities lying near the extreme tip of the posterior end and giving rise to an open, porous structure. The longitudinal vessels appear to connect with them, as do also the vessels of the peripheral layer, of which they appear to be enlargements. In sections approaching from the opposite direction there were two elliptical spaces, the long axes of which are parallel with the long axis of the body. One of these is shown highly magnified in plate XXVI, fig. 5. These lay side by side and each appeared in about eleven of the sections. The thickness of the sections was about .015 millimeter. They were completely inclosed in the tissues of the body except at the posterior ends where each communicates with the exterior by a short passage with nuclear walls. When the sections are studied with a view to reconstructing the cavities the latter are seen to be ellipsoidal, or more properly, lenticular. These spaces have a special lining of columnar epithelium resting on a nuclear layer and surrounded by a muscular layer which is made up of the continuation of the inner layer of longitudinal muscles. The latter layer also contains numerous nuclei. The maximum length of these spaces is .28 millimeter, the breadth .20 millimeter, and the thickness (estimated) .02 millimeter. They are filled with loose, granular tissue and delicate connective fibers somewhat like the tissue which fills the marginal canals, but differing from that in that the granular material takes a good stain with carmine. There were about fifty sections made through the posterior end of the specimen. The sections of these two oval vessels did not begin nor end at the same place in the series, although in a number they occur side by side. One occupies about seventeen sections; in about half of these it is accompanied by the other.

A third vessel with the same structure, viz, thick walls of columnar tissue appears in a few of the sections near the lateral face opposite the one near which the two above-described vessels lie. It occupies about six sections, is much smaller than the others, and the walls, instead of being smooth, are much folded.

I interpret these vessels as the terminal pulsatile vessels. I was not able, from the single series of sections prepared for this preliminary report, to determine their exact relations to either the aquiferous vessels or the marginal canals.

*Calcareous bodies.*—These are not abundant in the extreme anterior end of the body, but soon become very abundant as one proceeds posteriorly from the head. As in *L. catostomi* they are confined mainly to the peripheral granulo-vascular layer and to the central core. In the latter they are very abundant. They are somewhat larger and present rather more irregularity in shape than do those of *L. catostomi*, and finer examples of concentric structure occur. The dimensions of one of the larger bodies were .011 millimeter and .019 millimeter in the two diameters. They are seen to be still abundant, particularly in the central core, in sections made through the posterior end.

*Reproductive organs.*—The reproductive organs are but slightly developed. The beginnings of the cirrus pouch and the vagina are indicated by a series of clusters of nuclei which lie along the middle line near one of the lateral faces. No other traces of genitalia were observed.

## YOUNG FORMS ENCYSTED IN ABDOMINAL CAVITY.

[Pl. XXVII, Figs. 1-4 and 6.]

Larvæ of this species occur not only in the abdominal walls, but also in cysts in the peritoneum of the stomach and intestines, particularly among the pyloric cæca, occasionally in the liver and spleen. Some of the cysts in the trout submitted to me for examination contained embryos 4 centimeters and over in length. These were coiled up within the cyst in irregular folds, and in places the bodies were much constricted. One specimen whose average breadth was from 1 to 1.5 millimeters was constricted in one place to a diameter of .5 millimeter. This specimen, which was about 4 millimeters in length, was similar in general outline to the larger specimens from the abdominal wall. Like them the body was crossed with fine transverse lines.

Since these specimens frequently break at the narrow constrictions, many of the alcoholic specimens present a deceptive appearance, looking as if the larva might be a worm with a slender neck, such as may be seen in such worms as *Dibothrium rugosum*, whose anterior end is often fixed firmly in the tissues of its host and there degenerates into a slender core. Some of the cysts contained a waxy secretion with a calcareous mass at the center, but no parasite. These are evidently cases of arrested development where the tissues of the parasite have undergone degeneration. One cyst 9 millimeters long and 5 millimeters broad after the connective tissue layers had been removed, when opened revealed an embryo which measured, approximately, 2.75 centimeters in length and was 1.5 millimeters wide at the widest point. The body was extremely irregular and had several constrictions. These were most pronounced near each extremity. On each side of a constriction the body swells out abruptly, producing an effect like that of a chain of small tubers. This phenomenon is doubtless due to the fact that much of the parenchyma of the body was in a plastic condition, and when the specimen was placed in alcohol the result of the unequal contraction of different parts of the body, together with its cramped and confined condition, was to impress this characteristic outline on the parasite. A small specimen about 5 millimeters long was obtained from a cyst 7 millimeters in diameter. In the center of the cyst was an amber colored, rather hard mass, which effervesced slowly with dilute hydrochloric acid. Another specimen, 8 millimeters long, folded once on itself, completely filled its cyst. This specimen was 2 millimeters broad, posterior end truncate, slightly emarginate, anterior end bluntly rounded. In some cases the embryos were embedded in the parenchyma of the cyst. In nearly every case the embryos were associated with a comparatively large mass of the original food-stuff. The cyst is evidently a nurse or blastocyst, with an investment of connective tissue, and the embryo has developed by a process of budding within the blastocyst. The embryos are flask-shaped—larger at one end than the other—gradually tapering to the smaller end. Near the larger end they are distinctly shouldered. This character is not present when the extremity is invaginated. Beyond the shouldered part there is a somewhat narrowed prolongation with a slit on each lateral face. These slits appear to be rudimentary bothria. The cysts are, for the most part, irregularly globular or oval. One, however, was observed that was elongated; in the latter the contained embryo was straight.

*Anatomy.*—One blastocyst containing an arcuate embryo curved around a ball of hardened parenchyma, and two embryos which had been liberated from their blastocysts were stained with borax carmine and cut into sections.

The musculature and vascular systems are substantially the same as in the larger specimens. There is, however, an epidermal layer present (plate XXVII, fig. 6, *a*) which was absent from the larger specimens. This epidermis appears in transverse sections as a border of short, curved, hair-like processes springing from a thin basement layer, which separates easily from the cuticle. The remaining layers are much like those of the larger specimens, except that the inner circular layer and the radial fibers are not yet differentiated. The layers named from the outside toward the center are: (1) the epidermal layer, (2) a thin, structureless cuticle, (3) a narrow layer of fine longitudinal fibers, (4) a dense nuclear layer, (5) a comparatively broad layer with scattered nuclei and a few calcareous bodies, and the vessels of the peripheral system, (6) a rather narrow but very persistent and strongly marked layer of longitudinal muscles. Within the layer of longitudinal muscles there is no further differentiation into layers. In it, however, a net-work of fine connective fibers, with numerous nuclei, a few calcareous bodies, the aquiferous vessels and marginal canals, are clearly revealed in the stained sections. In some of the sections a slight tendency to parallelism in the connective fibers immediately within the layer of longitudinal muscles indicated the beginning of the layer of circular muscles which occurs at this place in the larger forms.

The marginal canals and the aquiferous vessels have essentially the same structure as the same vessels in the larger specimens.

The principal aquiferous vessel lies rather closer to the marginal canals than is the case in the larger specimens. As in the larger specimens, the marginal canals are characterized by their relatively large size, absence of proper walls, abundance of nuclei surrounding them, and a spongy, unstained interior tissue. Close to the central border of each of the marginal canals is the smaller aquiferous vessel (plate XXVII, fig. 6, *g*), with proper wall, open lumen, and in longitudinal section an irregular, crumpled, and interrupted outline marking its tortuous course. Its wall is surrounded by nuclei.

There are but few calcareous bodies in the central core. They are smaller and more uniformly elliptical in outline than those of the larger specimens. The largest measured .012 by .008 millimeter in their two optical diameters.

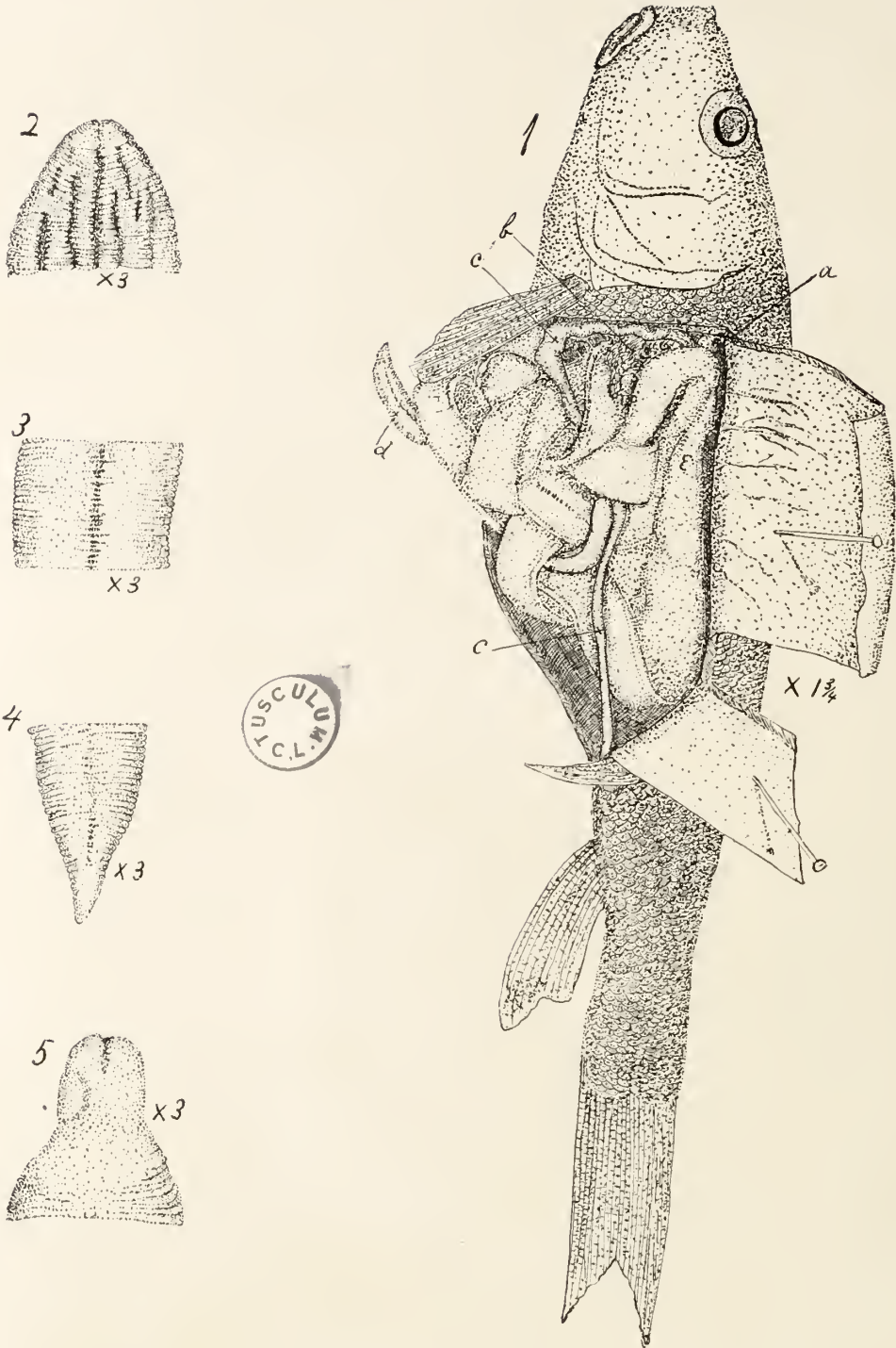
The blastocyst has the following structure: Its investing wall consists of a number of concentric layers, which in section appear like parallel fibers. These layers are rich in nuclei. In some of the sections in the vicinity of the embryo, these had become somewhat separated from each other, and as many as sixteen were counted. These layers appear to result from the delamination of the outer portions of the parenchyma of the blastocyst. Nuclei exist in abundance in the external portion of the mass of parenchyma on the side adjoining the embryo.

For the most part the parenchyma appears uniformly granular and non-nucleated. There is some evidence, however, of both contractile tissue and vascular structures.

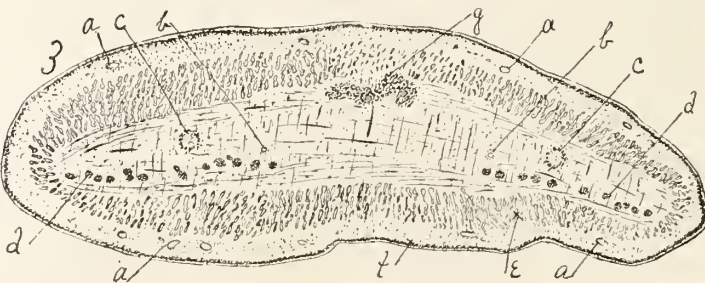
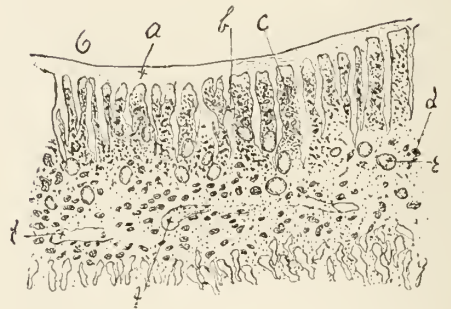
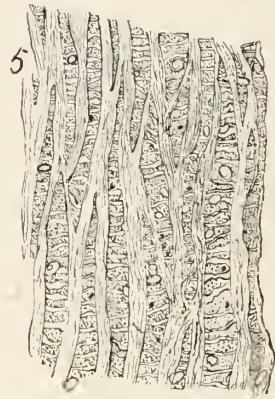
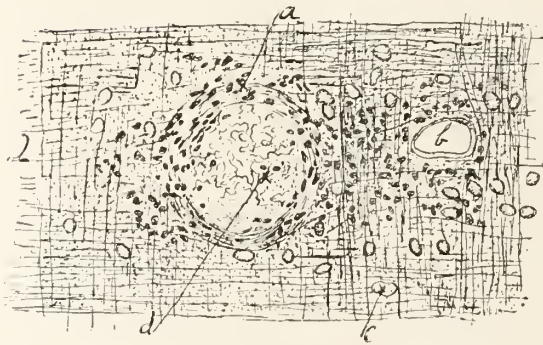
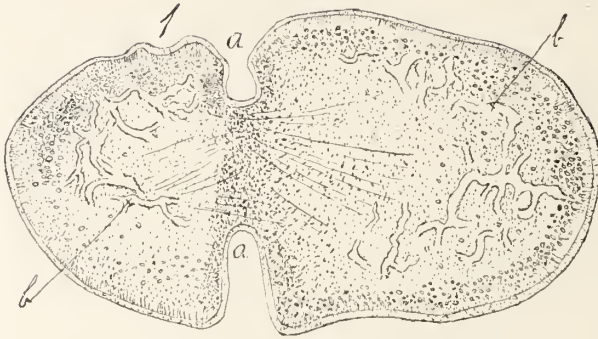
Several *Nematods* from these pyloric caeca, where they were encapsuled in the serous membrane, a few also in cysts in the peritoneum, and one *Echinorhynchus* were found in these trout. It has been thought best not to include descriptions of them in this paper.

WASHINGTON, PA., February 27, 1890.











## EXPLANATION OF PLATE XXIII.

- Fig. 1. *Ligula catostomi* in abdominal cavity of sucker (*Catostomus ardens* J. & G.).  
*a, b.* Viscera of host. The heart, liver, spleen, etc., are crowded into a very small space in the anterior dorsal part of the body cavity.  
*cc.* Intestine of host.  
*d.* Posterior end of Ligula.  
*e.* Anterior end of Ligula.  
 Fig. 2. Anterior end of Ligula.  $\times 3$ .  
 Fig. 3. Median region of body.  $\times 3$ .  
 Fig. 4. Posterior end.  $\times 3$ .  
 Fig. 5. Anterior end of another specimen.  $\times 3$ .  
 Drawings by the author.

## EXPLANATION OF PLATE XXIV.

- Fig 1. *Ligula catostomi*. Transverse section near anterior end.  $\times 60$ .  
*aa.* Bothria.  
*bb.* Peripheral aquiferous vessels.  
 Fig. 2. Transverse section of longitudinal vessels near anterior end.  $\times 300$ .  
*a.* Circular fibers with nuclei surrounding one of the marginal canals.  
*b.* Lumen of aquiferous vessel.  
*c.* Calcareous body.  
*d.* Nuclei in spongy tissue forming lumen of marginal canal.  
 Fig. 3. Transverse section near posterior end of body.  $\times 27$ .  
*aaaa.* Peripheral aquiferous vessels.  
*bb.* Central aquiferous vessels.  
*cc.* Marginal canals.  
*dd.* Testes.  
*e.* Longitudinal muscle layer.  
*f.* Subenticular granular layer, which, when highly magnified, shows the beginning vitellaria.  
*g.* Incipient generative organs.  
 Fig 4. One of the nests of nuclei forming a testicle *d* of Fig 3.  $\times 750$ .  
 Fig. 5. Longitudinal section through longitudinal muscle layer from median region of the body, enlarged about 300 diameters. The section was made parallel to a lateral face. A few nuclei and calcareous bodies are shown. The latticed appearance of the interstitial spaces is due to the radial fibers mentioned in the text.  
 Fig. 6. Portion of the border of a transverse section from same region as Fig 2. Enlarged about 300 diameters.  
*a.* Cuticle.  
*b.* Plate of peripheral longitudinal muscle layer.  
*c.* Granular and nuclear protoplasm.  
*d.* Nuclei of vascular layer.  
*e.* Calcareous bodies.  
*ff.* Peripheral aquiferous vessels.  
*g.* Outer portion of inner longitudinal muscle layer.  
 Drawings by the author.

## EXPLANATION OF PLATE XXV.

- Fig. 1. *Ligula catostomi*. Portion of transverse section from same region as Fig. 3 of Plate 4. Enlarged about 60 diameters.  
*a.* Cuticle.  
*b.* Remains of external longitudinal muscle layer.  
*cc.* Peripheral aquiferous vessels.  
*d.* Calcareous bodies.  
*ee.* Longitudinal muscle layer.

- f. Circular layer.
- g. Rudimentary male generative organs.
- h. Rudimentary female generative organs.
- i. Position of genital aperture.
- k. Subcuticular granular layer, vitellaria.

Fig. 2. *Dibothrium cordiceps*. Transverse section near anterior end.  $\times 75$ .

- aaa. Peripheral aquiferous vessels.
- bb. Central aquiferous vessels.
- cc. Marginal canals.
- d. Row of nuclei connecting marginal canals.
- ee. Bothria.
- f. Epidermal layer.
- g. Nerve cells?

Fig. 3. Longitudinal section near margin. (The outer layer of longitudinal muscles is wanting near the margins, the space being occupied by masses of nuclei which have much the same appearance in longitudinal sections as they have in transverse sections.)  $\times$  about 250 diameters.

- a. Cuticle.
- b. Subcuticular granulo-nuclear layer.
- cc. Peripheral aquiferous vessels.
- d. Calcareous body, showing concentric structure.
- e. Longitudinal muscle layer.
- f. Nuclei of vascular layer.

Fig. 4. Calcareous body, optical section showing concentric structure.  $\times$  about 750 diameters.

Fig. 5. Portion of abdominal wall of *Salmo mykiss* with parasite inclosed.  $\times 3$ .

- a. Epidermis of host.
- b. Muscular tissue of host.
- c. Connective tissue layer of cavity in which the parasite lies.
- d. Posterior end of parasite.
- e. Anterior end of same.

Drawings by the author.

#### EXPLANATION OF PLATE XXVI.

Fig. 1. *Dibothrium cordiceps*, anterior end.

Fig. 2. Middle.

Fig. 3. Posterior end. Figs. 1-3,  $\times 6$ .

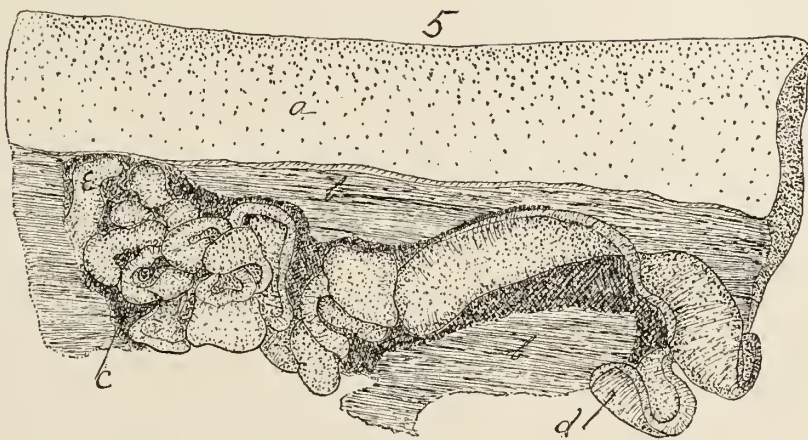
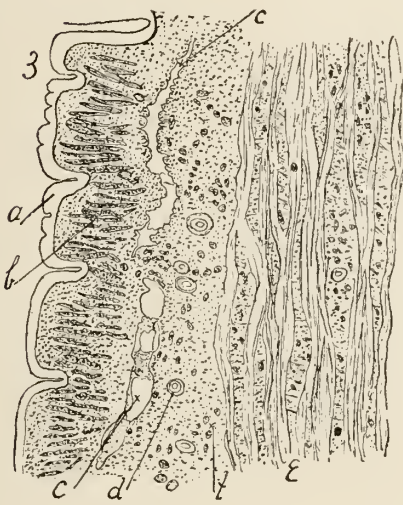
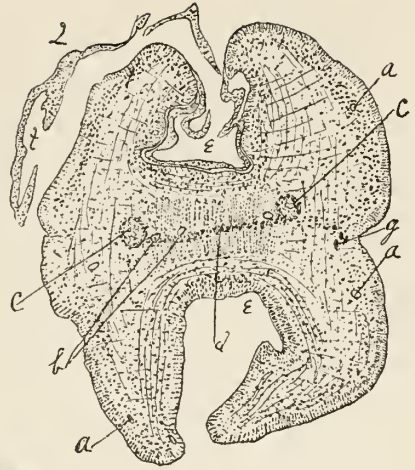
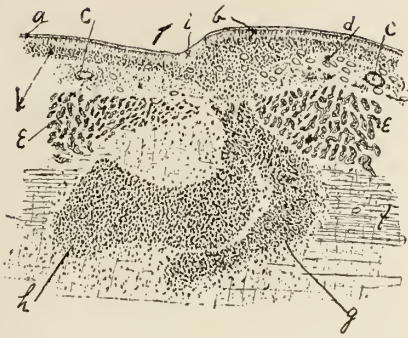
Fig. 4. Transverse section of longitudinal vessels, near anterior end.  $\times$  about 200 diameters.

- aa. Cut end of longitudinal muscles.
- bb. Circular muscles.
- cc. Calcareous bodies.
- c'c'. Same in optical section, showing concentric structure.
- d. Marginal canal.
- e. Central aquiferous vessel.

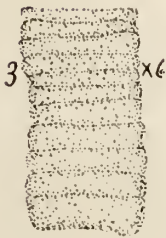
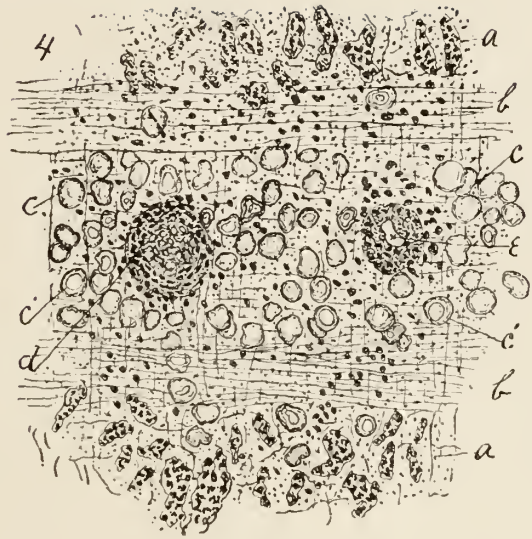
Fig. 5. Longitudinal section of pulsatile vessel posterior end.  $\times$  about 200 diameters.

- a. Layer of columnar epithelium.
- b. Excretory pore.
- c. Cuticle.
- d. Subcuticular layer of nuclei.
- e. Parenchyma with connective tissue and nuclei.
- f. Nuclei of muscular layer surrounding pulsatile vessel.
- f'. Granulo-nuclear layer supporting columnar epithelial layer.
- gg. Longitudinal muscle fibers.
- h. Cluster of nuclei marking first appearance of second pulsatile vessel.

Drawings by the author.

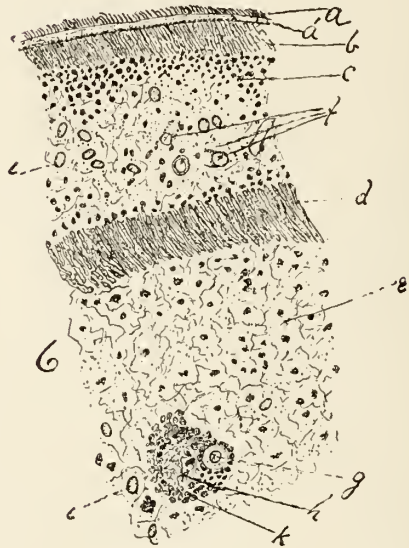
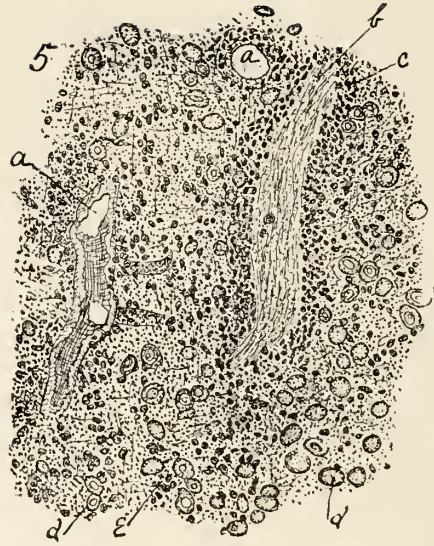
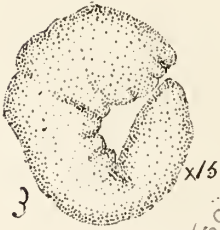
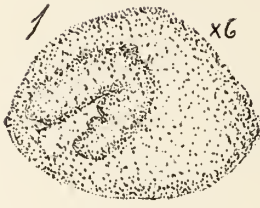














## EXPLANATION OF PLATE XXVII.

Fig. 1. Cyst containing embryo from pyloric cæca of *Salmo mykiss*.  $\times 6$ .

Figs. 2, 3, 4. Embryos liberated from their cysts.  $\times 15$ .

Fig. 5. Longitudinal section of longitudinal vessels of specimen from abdominal walls of host; sections from same region of body as that of Fig. 4, Pl. IV.  $\times$  about 300.

*aa.* Spaces where aquiferous vessel is cut through; the vessel is seen to have both longitudinal and circular muscle fibers in its walls.

*b.* Lumen of marginal canal; the spongy tissue which fills it has a striated appearance in longitudinal section.

*c.* Nuclei.

*dd.* Calcareous bodies.

Fig. 6. Part of transverse section of embryo from cyst in pyloric cæca, highly magnified.

*a.* Epidermis.

*a'.* Cuticle.

*b.* External layer of longitudinal muscles.

*c.* Nuclei.

*d.* Inner layer of longitudinal muscles.

*e.* Parenchyma, with connective tissue fibers and nuclei.

*f.* Peripheral aquiferous vessels.

*g.* Central aquiferous vessel.

*h.* Marginal canal.

*ii.* Calcareous bodies.

*k.* Nuclei surrounding marginal vessel.

Drawings by the author.

