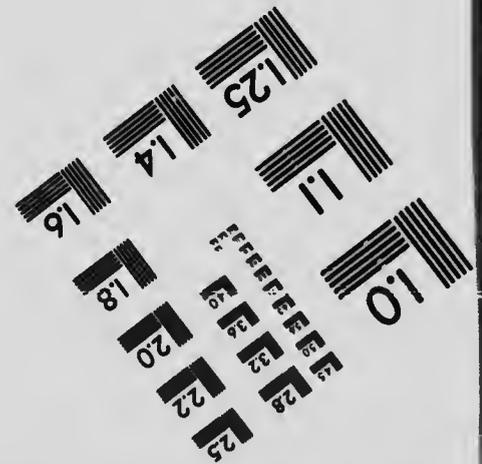
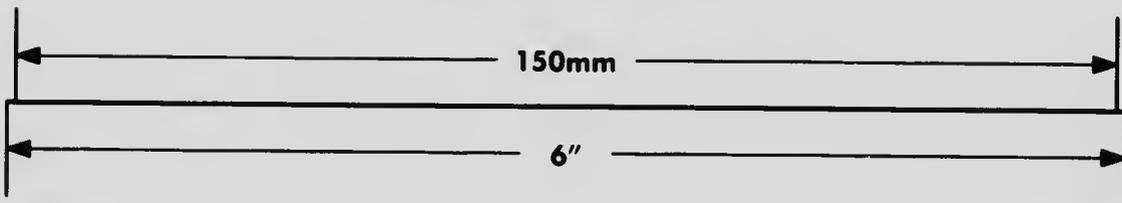
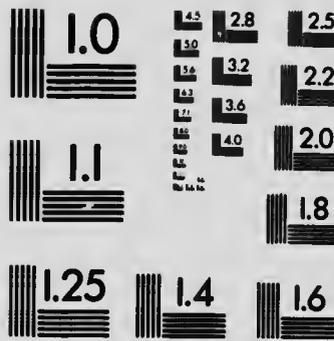
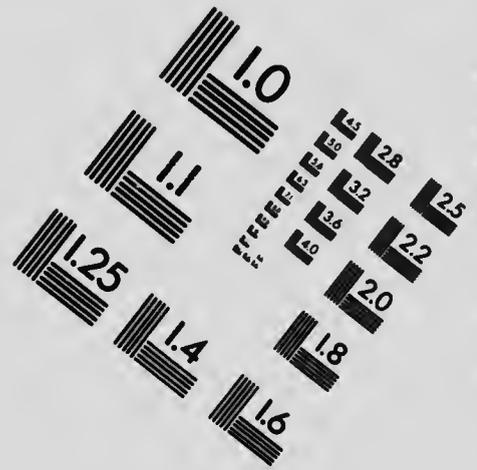
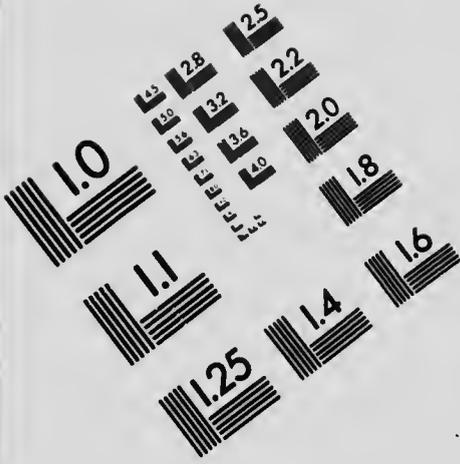


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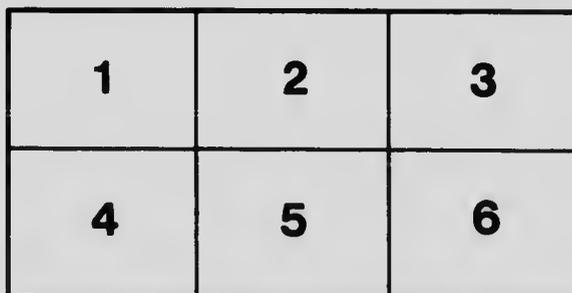
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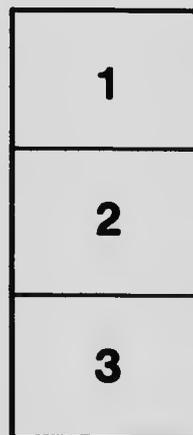
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BIOLOGICAL SERIES

No. 15: A NEW CESTODE FROM *AMIA CALVA* L, BY A. R.
COOPER

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A NEW CESTODE FROM *AMIA CALVA* L.

BY A. R. COOPER, M.A.,

(Read 26th October, 1914)

A few years ago, Professor R. R. Wright drew the writer's attention to a Bothriocephalid which, during the course of his earlier helminthological researches, he had found in *Amia calva* L. and believed to be entirely new. Later specimens of the same genus, and perhaps, too, of the same species, were procured from the same host taken in the vicinity of the Lake Biological Station on Georgian Bay; and, since a preliminary examination showed that the worm had apparently not yet been described, it was thought advisable to make it the subject of a more or less thorough investigation, and to publish the results.

The writer wishes to herewith express his indebtedness to Professor B. A. Bensley for valuable assistance and advice in connection with the preparation of this paper, and to Professor H. B. Ward, of the University of Illinois, for opinions on a preliminary description and for material from his private collection.

The following paper is concerned only with the morphology of the worm, a consideration of its systematic position having been dealt with in a second paper published in the Transactions of the Royal Society of Canada (Series III, Vol. VIII, 1914, pp. 1-5).

MATERIAL.

Apart from a few examples kindly sent to the writer by Dr. Ward, the material consists of worms ranging in length from a few millimeters to about ten centimeters, taken from the duodenum of three or four specimens of *Amia calva*, L. These were all fixed in Alcoholic-acetic-sublimate*, and stained in bulk for transparency-preparations with Meyer's Acid Carmine and in sections with Heidenhain's Iron-Haematoxylin and Orange G or Mallory's stain, the latter to bring out basement membranes in particular.

GENERAL APPEARANCE.

When removed from the anterior end of the intestine of the host to normal saline solution the cestodes are quite active, undergoing changes in length and breadth particularly in the middle and posterior portions of the strobila; those in the scolex and most anterior proglottides are less

*The Taenioid Cestodes of North American Birds, by B. H. Ransom; Proc. U.S. Nat. Mus., Bulletin 69, 1909.

extensive. When greatly extended they appear somewhat thread-like to the unaided eye, when contracted, during life or after preservation, if no care has been taken to stretch the specimens, somewhat like a string of fine beads, which characteristic has been incorporated in the specific name. This is due anteriorly to the thickened hinder ends of the foremost joints, while farther back it is caused by the uteri being greatly distended with eggs.

The largest specimens examined were two, 110 and 96 mm. in length, containing respectively 59 and 55 proglottides. From uncleared material the number of the latter is obtained by merely counting the joints forward and depending on the distension of the uteri—the male and female genital openings are very minute—in the hinder end of the strobila to indicate the sets of reproductive organs, there being no other in evidence of proglottidation in this region.

The scolex is quite small, simple externally, and with the unaided eye can scarcely be distinguished from the first joints. It is shaped roughly like a rectangular solid, hollowed out laterally to form simple depressions and dorso-ventrally the shallow bothria or organs of attachment. The summit is somewhat prolonged as a low pyramidally shaped disc, quite comparable to that ("Scheitelplatte") found in the members of the sub-family *Triaenophorinae* Luehe, 1899. Furthermore, although to all outward appearances this structure is unarmed, certain modifications of the cuticle on the edges, as well as on those of the foremost proglottides, to be described below, strongly remind one of the minute hooks with which *Ancistrocephalus microcephalus* (Rud.) is provided. The opposite end of the scolex is modified to form two pairs of auricular appendages closely resembling internally as well as externally those of the foremost joints (Fig. 1). The following measurements of scolex will be of use for future diagnoses of species:—

Width, at base of terminal disc.	0.20 — 0.40 mm.
Width, at posterior end of bothria.	0.17 — 0.25 mm.
Width, at tips of appendages.	0.24 — 0.38 mm.
Length, including appendages.	0.38 — 0.48 mm.

A neck is absent, proglottidation beginning immediately behind the scolex (Figs. 1, 5 and 6). Here the joints are short and crowded closely together even in relaxed states of the strobila. The appendages are united to form a sort of ring into which the narrow anterior end of the next joint fits, leaving recesses between these two parts, which pass forward a little farther laterally and dorsoventrally than at the ends of the diagonal diameters (Fig. 35). In many preserved specimens these

appendages with those of the terminal disc stand out as thin leaf-like structures, concaved anteriorly, thus suggesting their probable use as accessory organs of attachment ("Stützorgane") to the wall of the host's intestine. The bothria, although provided with a well-developed musculature (vide infra), would seem to be incompetent to securely fasten the worm; possibly the appendages of the scolex and foremost proglottides may combine to act as temporary suckers, as suggested by several authors. Unfortunately no observations on the methods of attachment were made on the living animals.

On passing backwards, the joints are seen to elongate considerably, especially in all parts ahead of the ring of appendages which remain relatively more constant in size. A transverse section through the former is oblong in shape, while one through the latter is more broadly elliptical to circular in outline. This part of the strobila is the most mobile, elongation often reaching the degree mentioned above in which the appearances are quite like a knotted thread. Fig. 2 shows different degrees of contraction in a portion of the chain, but it can be seen that the middle joint is naturally somewhat shorter than the other two. In many chains this region is subject to considerable variation. It was observed that now and then one of the longest proglottides was provided with one or two additional pairs of appendages, generally abortive and situated anteriorly some distance apart. In a few cases staining and clearing brought out a distinct division of the parenchyma, especially posteriorly, into what seems to be the beginnings of a division of the longer proglottis into several smaller ones. Furthermore in one strobila an undivided region was intercalated between two jointed regions, the second of which was followed by the normal posterior end. Young scolices are shown in Figs. 5 and 6. (In this connection note evidence given below under the excretory system that the latter are incomplete). Although the foregoing facts point to possibly occasional augmentation in the number of proglottides in this region in adult worms, the usual appearances are as described below.

Beginning at the 15th to 17th, the proglottides enlarge somewhat abruptly until the size shown in Fig. 3 is reached. The dotted ovals here represent the gravid uteri which give rise to the distended appearances of the posterior two-fifths, or nearly, of the joints. There is also some increase in width anteriorly. On the other hand the auricular appendages gradually diminish in size, until after the 23rd or 24th joint they are not to be seen, the strobila then resembling a ribbon swollen at regular intervals, as mentioned above. For some distance farther the remains of the constrictions of the anterior ends of the joints are seen in slight approaches of the lateral borders, while still farther back a tendency

for material cleared in oil of cedar, which is very brittle, to break immediately behind the ovary is the only other indication, apart from the reproductive organs, of proglottidation. This tendency, however, so far as could be determined is not based on any differentiation of the parenchymatous tissues internally at this level but more probably on mere differences of support in the latter, the ovary rendering the parts immediately ahead more resistant to strain. The following are some relative measurements of a typical strobila (Fig. 3):—

Proglottis.	Length.	Greatest Width.
20	1.85 mm.	0.48 mm.
22	2.37 mm.	0.48 mm.
24	2.03 mm.	0.58 mm.

What is apparently the end-proglottis is rounded posteriorly (Fig. 12) and provided with a functioning set of genital organs. The endings of the excretory vessels in this joint, however, seem to point to some part of the strobila (perhaps, also, of the plerocercoid) being lost at an early stage (*vide infra*).

CUTICULA.

The cuticle, a well-developed structure excepting in the oldest portions of the strobila where it is often much torn or even missing over small areas, is from 3 to 4 μ in thickness. It is divisible into two principal layers in each of which other layers can be distinguished. The outer of these, about two-thirds as thick as the inner, does not stain as well as the latter owing to the fact that it is made up of alternating dark and lighter areas arranged so as to give a striated appearance. The darker lines seem to be composed of minute granules while the lighter are more homogeneous (Fig. 7). Bounding this layer peripherally there is to be seen in many sections an extremely narrow clear line, followed by a sort of external limiting membrane, while in others, especially those through young strobilas, only very minute teeth which seem to be continuations of the darker lines are visible. The inner layer of the cuticula takes stains much more readily than the outer and is quite homogeneous with the highest magnifications. The line separating the two, however, is slightly darker than even the inner, which is perhaps due either to larger granules than those in the dark lines of the outer layer or a greater number packed more closely together. Bounding the inner layer on the inside there is a well-developed basement-membrane, brought out best by Mallory's stain. This is often separated from the homogeneous layer by a clear line as indicated in the figure. Then again just outside the basement-membrane the former is slightly granular in some quite thin sections. The cuticle is traversed at short intervals by the minute

excretory canals forming the foramina secundaria which appear in tangential sections as circular openings in a homogeneous matrix. Since these course through the cuticle quite obliquely, they give the latter the appearance of being pierced with holes at different levels. Two of them are shown in Fig. 7, one having reached the outside while the other has not yet passed the basement-membrane.

In many cases a splitting of the outer layer of the cuticle into processes takes place evidently along the lighter striations. It is quite conceivable that the cuticular processes, if not "cilia", described for many *Bothriocephalids* may arise in this manner in young scolices.

The cuticle covering the scolex is, on the whole, somewhat thinner than that on the posterior proglottides. This statement is also applicable to that on the inside of the auricular appendages of the scolex and foremost joints. The other modification of the cuticle, referred to above, is best seen in young scolices where the minute spines have not been worn away. It will be seen, by reference to Fig. 8, that the latter are developed as a thickening in the outer layer followed by a breaking up of the material into stout spine-like processes. These minute spines are restricted to a very narrow line running along the edge of the auricle, and are all directed towards the inner concave surface of the latter, that is, towards the central longitudinal axis of the worm. They gradually disappear with the appendages posteriorly. Since these spines appear in great numbers, and, since the appendages are provided with well-developed sets of muscles (v.i.), obviously arranged to activate them, they must be of actual service to the worm in obtaining a hold on the smooth mucous lining of the host's intestine.

SUBCUTICULA.

The subcuticular cells (Fig. 7) are not clearly defined as to boundaries but are fused together to form a syncytium the extent of which is indicated chiefly by the nuclei. There are, however, condensations of protoplasm around the latter in ripe proglottides, giving the appearance of columnar cells which have been described for many *Bothriocephalids*. These may even be more or less distinct towards the centre of the proglottis, yet they are directly continuous with processes from the cells of the parenchymatous tissue beneath, the whole forming in many places a meshwork of protoplasmic strand surrounding vacuoles, as shown in the left of the figure. The nuclei are comparatively large structures with well-defined walls, non-uniform in thickness, and clear contents, excepting for the deeply-staining "nucleoli". The thickness of the subcuticula varies in different regions, especially since its inner boundaries are rather indefinite, averaging about 25μ . Numerous processes proceed towards

the cuticula, beyond the basement-membrane of which they could not be traced. The space between the latter and the circular cuticular muscles stains less deeply, since there seems to be a condensation of protoplasm into strands which traverse it. In some places, in sections of young strobilas, these processes appeared to be more or less grouped opposite the columnar condensations in the syncitium, mentioned above. The subcuticula is poorly developed in the scolex.

PARENCHYMA.

The parenchyma is divisible into the usual parts, a medulla ("Markschicht"), at the centre of the strobila, surrounded by a cortex ("Rindenschicht"), extending to the subcuticula, the two being separated by the longitudinal muscles. This division into two parts is based rather on the arrangement of the nuclei, since the cytoplasm forms a very open reticulum, excepting immediately around the nuclei (Fig. 35), in which cell-boundaries cannot be seen. In the anterior proglottides most of the nuclei, each from 4 to 5 μ in diameter, are restricted to two regions, those of the medulla close around the excretory vessels and nerve strands, those of the cortex, much more numerous than the inner lot, close to the subcuticula and among the outer transverse muscles. The myoblastic nuclei of the transverse (inner especially) and dorso-ventral muscles are easily confused at first sight with the nuclei of the parenchyma, but on closer examination they are found to be somewhat smaller and to contain more chromatin granules, a distinct nucleolus being difficult to locate. The nuclei of the parenchyma, on the other hand, are slightly smaller than those of the subcuticula. In Fig. 7 the two smallest nuclei farthest from the cuticle doubtless belong to the peripheral region of the cortex.

In young strobilas the parenchymatous reticulum is very vacuolated, being indicated mostly by granules at the intersections of fine protoplasmic strands, while in mature proglottides it is evidently used up for the growth of the reproductive organs which fill up almost the whole space within the subcuticula.

At the summit of the terminal disc of the scolex very many nuclei are crowded around the small shallow depression to be found there in many specimens, but they show no evidence of having any special function. Probably they have been pushed out of the immediate neighbourhood by the growth of the powerful muscles situated there (v.i.).

In all of the material studied there appeared to be no traces of chalk bodies in the parenchyma, not even spaces such as can be seen in plerocercoids of the genus *Proteocephalus*, which might have accommodated them before they were dissolved out by the acetic acid in the fixing fluid.

MUSCULATURE.

The musculature consists of two series of fibres, namely, the muscles of the parenchyma, coursing in three different directions, and those of the cuticle, which are closely related through what will be described below as the outer longitudinal group of the former. Since a careful study of the muscles was made, they will be dealt with somewhat in detail, beginning with the simplest histologically, the dorso-ventral and coronal fibres. Those of the scolex will be described separately.

In his researches on *Bothridium pithonis* Blain. Roboz ('82) was unable to find the myoblastic nuclei of the longitudinal muscles, which, he says, are pointed at both ends, but observed a longitudinal fibrillar striation. Zerneck ('95), working on several species, makes the following statements concerning the individual muscle-fibres: "Hier finden wir denn auch die von Salensky für die Muskeln von *Amphilina* beschriebene Differenzirung der Fasern in eine centrale (Mark-) und eine periphere (Rinden-) Schicht. Letztere umgiebt den centralen Theil als ein breiter Ring und ist von diesem durch die intensive Färbung zu unterscheiden. Sie ist von homogener Structur und starker lichtbrechend als das Centrum. Letzteres erscheint im Querschnitt als eine dunklere, feinkörnige plasmatische Markmasse"; further, "Hier (at the level of the myoblastic nucleus) ist der Zusammenhang der Marksubstanz mit der Zelle zu sehen. Die Rindenschicht bildet hier nicht mehr ein geschlossenes Rohr um die Markmasse, sondern öffnet sich an einer Seite, so dass eine Rinne entsteht, durch welche das Plasma der Bildungszelle mit dem Mark communicirt." Essentially the same conditions were found in the musculature of this form, excepting that the peripheral layers of the individual fibres of all of the different groups are characterized by being broken up into a varying number of fibrils (Figs. 10a, b, c, and 9a) which diverge at the ends, excepting in the case of the longitudinal fibres of the parenchyma. An example from the coronal series (Fig. 9a) shows how the fibrils are related to the nucleus and cytoplasm. Although in most of the fibres of the longitudinal muscles the latter are situated close to the fibrils, as shown in Fig. 9d, others, Fig. 9b and c, are widely separated from them, the connection being scarcely visible in many cases. The two figures given are of the most distinct examples that were seen. The fibrils themselves are very easy to follow in every part of the strobila. In cross-sections of the external longitudinal fibres at certain levels, a large area of highly-staining material at one side of the fibre (Fig. 10a and b) was considered to be the remains of the nucleus, since no other trace of it was found. This and the fact that the myoblastic nuclei of the dorso-ventral fibres between the bothria were somewhat degenerate and quite closely related to the

fibrils (Fig. 10c) points to a specialization among many sets of fibres in the direction of the complete loss of the nuclei, the presence of which is made the basis of a classification of the muscles of cestodes by Braun ('94-'00).

The coronal or transverse series of muscles is arranged as two thin sheets of fibres lying immediately within the longitudinal muscles of the parenchyma, thus assisting the latter in forming the boundary between the medullary and cortical parenchyma. In transverse sections the fibres of these two layers diverge laterally so that the innermost cross or interdigitate before they become attached to the cuticle. In the posterior end of the anterior proglottis each layer sends many fibres to the auricular appendages of the same surface of the worm (Fig. 35), which curve slightly posteriorly to form part of the radiating fibres of the latter. While the dorsal and ventral bands are continuous from joint to joint throughout the anterior portion of the strobila, there is a decided augmentation in the number of fibres in the posterior portion of the proglottis opposite the auricles into which they pass. Farther back they diminish in number with the reduction in the size of the appendages, relatively more quickly in the forward part of the joint, until in the unsegmented hinder end only a few straggling fibres appear, in the interproglottidal regions, between the testes and the vitelline glands.

In addition to these another series of transverse fibres, more circularly arranged, appears in the anterior end of the strobila, especially well-developed in the first three or four joints. They are divided into eight groups, two for each surface, and are situated immediately beneath the subcuticula. Each group consists of parallel fibres arising along the whole of the edge of the proglottis ahead of the appendages and passing obliquely and posteriorly into the opposite appendage of the same surface. Thus there is a decussation in the mid-line, giving rise to rather complicated appearances in cross-sections. The two pairs of lateral groups which can be best seen in sagittal sections, are related to each other in exactly the same way; they are, however, not quite so extensive, as may be expected from the ligulate habit of the worm, the dorso-ventral diameter even in these foremost joints being considerably less than the transverse. Beginning at the base of the appendages, that is, the anterior edge of the ring (vide supra) to which they are attached, small groups of these coronal muscles are cut off from the inner groups at the ends of the diagonal diameters of the joint by the external longitudinal fibres of the parenchyma to pass back into the appendages and supply them with a circular musculature (Fig. 35). All of the oblique fibres gradually disappear with the auricles posteriorly, so that they are developed evidently

for the movements of the auricles alone. From their arrangement they doubtless serve, in conjunction with other fibres to be described below, to extend the appendages away from the body as the leaf-like structures mentioned above.

The dorso-ventral or sagittal muscles are divided into six groups by the three excretory vessels and the two nerve strands which in the foremost joints occupy most of the medulla and are situated so close together in many sections that only individual fibres appear between them. The fibres themselves are more numerous, like the coronal muscles, anterior to the junction of two proglottides where the four most lateral groups, i.e., those between the nerve strands and the lateral vessels and those outside of the nerve strands pass from auricle to auricle on each side of the worm (Fig. 35). In the forward end of the joint more fibres are situated between the vessels and fewer laterally. The middle lot could not be traced beyond the subcuticula, while the lateral groups, on the other hand, can be easily followed to the cuticula of the auricular ring and appendages, in which latter they, along with the coronal fibres mentioned above, constitute the transversely radiating group. Farther back they dwindle down gradually until in gravid proglottides only a few coiled fibres appear between the testes and vitelline follicles or alongside the cirrus-pouch and uterine-cavity. The individual fibre closely resembles that of the coronal series, shown in Fig. 9a, excepting that it is shorter.

The longitudinal muscles of the parenchyma are divisible into two series, an inner and an outer, of which the latter appears only in the anterior end of the strobila. In transverse sections through the middle of the foremost joints they are arranged in small groups, with no constant number of fibres in each, in two concavo-convex bands between the medullary and cortical parenchyma, that is, about half way from the centre of the section to the periphery excepting laterally where they are situated relatively farther out. Here the thin edges come together immediately outside of the nerve strands. Throughout their course transversely they are penetrated by the sagittal muscles. As one nears the very short region between successive proglottides, in following through a transverse series, some of the fibres (more correctly fibrils, from the above view of the constitution of the fibre) decrease in diameter and number, especially laterally, and become more loosely arranged, as they diverge from one another. Immediately ahead or behind, as the case may be, they again appear as above. On the other hand a great many pass from joint to joint uninterrupted. From this fraying out of the fibres between successive joints it was concluded that the lengths of some of them, at least, did not exceed that of the proglottis: in the mature,

unsegmented portion of the strobila the question of the length is a very difficult one to decide upon. Perhaps only the longer kind is to be found here, since, as above stated, no indication of proglottidation, apart from the separate sets of reproductive organs, was detected. There is thus a certain amount of interruption in the course of the longitudinal muscles corresponding to the division into proglottides as pointed out early by Leuckart for *Taenia saginata* Goeze (Braun '00). Furthermore, there is a slight contraction of the whole cylinder of fibres as the interproglottidal space is neared (Fig. 37), which is not to be seen after the auricles have disappeared.

The outer longitudinal muscles appear only in the anterior proglottides and scolex in connection with the appendages, for the movement of which they are obviously developed. In the anterior half of the proglottis they lie very close to the longitudinal cuticular muscles from which they can be distinguished only by their slightly larger size. As they pass the slight indentation which in coronal series marks the anterior end of the appendage, they are joined by other fibres attached to the anterior portion of the outer wall of the latter, so that when they are converging towards the center to pass into the next proglottis, they form a ring of fibres more prominent in cross-section than those of the inner longitudinal group (Fig. 35). Throughout their course they are also more prominent opposite the auricles than at the ends of the dorso-ventral and transverse diameters. Just ahead of the sinus or pocket behind the auricle a few fibres are cut off from the main body to pass about half way along the inside of the appendage. The latter is further supplied with very many fibres belonging to the same group (Luehe '97) which pass between its outer and inner walls to the very tip (Fig. 37) and, by their contraction, obviously serve to protract the edge of the auricle and thus to allow the minute spines to catch in the mucous lining of the host's intestine. As the appendages diminish in size this series of muscles gradually becomes restricted to the hinder end of the proglottis and eventually disappears with the former.

Thus, so far as proglottidation in relation to the arrangement of the external longitudinal fibres is concerned, this species resembles *Ligula uniserialis* Rud. and strongly substantiates Luehe's generalization that: "wenn die Proglottiden eines Cestoden, wie dies in der Regel wenigstens bei jugendlichen Proglottiden der Fall ist, am Hinterende einen grösseren Querschnitt besitzen, als am Vorderende, dergestalt, dass die einzelne Proglottis mit einem seitlich abgeflachten Kegel verglichen werden kann und ein Langsschnitt durch mehrere Proglottiden eine der Schneide einer Säge ähnliche oberflächliche Begrenzung besitzt, so sind stets auch Muskelfasern vorhanden, welche an der Aussenfläche

der Proglottis entspringen und sich z. T. am Hinterende derselben den Bündeln der äusseren Langmuskeln beigesellen, z. T. an der freien Hinterfläche der Proglottis inseriren. Diese Muskelfasern werden nur dort vermisst, wo entweder eine äussere Gliederung fehlt oder die einzelnen Glieder nicht jene, für die jugendliche Proglottis charakteristische, regelmässige Form eines abgestumpften und seitlich abgeflachten Kegels besitzen."

The cuticular muscles are arranged in the unjointed portion of the strobila in the typical manner, while anteriorly they are modified somewhat in relation to the great development of the appendages. The outer cuticular fibres follow the cuticle closely (Fig. 7) even on the outside of the auricles and are diminished in number and size only opposite the bands of minute spines (Fig. 8). The longitudinal fibres, on the other hand, are largest and most numerous on the outside of the appendages to the tip of which they extend, while only a very few appear on the inside, being connected with those of the following proglottis after passing forward around the auricular pockets. This description applies also to the scolex in which, however, all of the cuticular musculature is not so well developed.

The musculature of the scolex is essentially the same as that of the anterior proglottides, but there are in addition two sets of fibres which do not appear in the latter. Furthermore, all of the muscles are better developed, that is, more numerous and larger, as one might expect in this portion of the strobila, specially differentiated for adhesion.

The coronal fibres are first seen about 70μ from the summit, after which they become more numerous, especially opposite the posterior boundaries of the longitudinal arcuate fibres (vide infra) then again opposite the appendages into which many of them pass as in the foremost joints. Their arrangement is shown in Fig. 34, a section through the middle of the scolex. The other series of coronal or transverse muscles in the scolex, the obliquely decussating group, are related to the auricles as are those in the proglottides immediately behind, excepting that they do not pass relatively so far forward at the edges or "walls" of the bothria (Fig. 36). A third series of transverse muscles, one of the two sets mentioned above, is composed of large fibres arranged concentrically towards the centre of the scolex from the edges of the bothrial walls (Fig. 34), which they protract, thus helping to deepen the bothria during attachment. They are situated in the middle third of the scolex, not extending beyond the limits of the bothrial depressions. These fibres interdigitate somewhat laterally and intermingle dorsoventrally with the attenuated edges of the sagittal fibres. They are quite homologous with the four groups

of fibres figured by Zograf ('92) for the scolex of *Triaenophorus nodulosus* (Pall.) and observed by the writer in confirmatory sections.

The sagittal fibres are arranged in the posterior portion of the scolex in quite the same way as those of the foremost joints. As you follow them forward, however, the two middle groups, that is, those between the excretory vessels, which separate somewhat to accommodate them (Fig. 34), enlarge considerably to form the chief muscles of the bothria. Contraction of these in conjunction with that of the tangentially arranged transverse fibres will deepen the bothria and thus form an efficient sucking-apparatus. By their relaxation and the contraction of the coronal fibres the bothria will, on the other hand, be loosened from the substratum. Anteriorly the dorso-ventral fibres gradually diminish in number and size until none appear in the first 70μ of the scolex from the summit.

The inner longitudinal muscles of the parenchyma do not pass to the tip as in many Bothriocephalids, but only about half way along the scolex, where they disappear.

The outer longitudinal fibres are arranged as in the foremost joints, but they are slightly more numerous. They extend forward as four groups each of which is situated near poorly developed cuticular muscles at the edge of the bothrial wall outside of the tangential groups (Fig. 34) ahead of which they do not appear, that is, they do not pass to the tip of the scolex.

The second group of muscles peculiar to the scolex only is to be seen in its anterior third. These are longitudinally arcuate fibres arranged concentrically around the edges of the terminal disc in four groups, one at each end of the diagonal diameters of the section through this region. Their function is obviously to contract the edge of the former with its bands of minute spines (Fig. 35).

The individual fibres of the transversely and longitudinally arcuate as well as those of the dorso-ventral bothrial muscles are comparatively short and spindle-shaped. Approximately their middle thirds take the strain much more readily (Figs. 34 and 37) than their ends which seem more muscular in composition and can be easily followed to the cuticle. This is due to the fact that it is in this middle portion that most of the cytoplasm and the nucleus are located (Fig. 10c).

The musculature of the end-proglottis bears out the above statement that an earlier portion of the strobila seems to be missing. The longitudinal muscles of the parenchyma dwindle down rapidly, the individual fibres diverging near the end-vesicle of the excretory system, while the cuticular fibres, excepting a few circular ones which pass farther on towards the latter, quickly disappear among the much altered subcuticular cells on the hinder border of the terminal joint (Fig. 12).

NERVOUS SYSTEM.

The nervous system consists of a nerve-ring situated immediately beneath the tip of the scolex and covering the median excretory vesicle (vide infra) like a cap, and the two chief strands passing back from it through the whole of the strobila. The former is a comparatively weakly developed structure (Fig. 11), elliptical in transverse section, with diameters of 60 and 40 μ . The chief nerve strands are 18 μ in diameter in the scolex, in which they are situated between the middle and lateral thirds of the medullary parenchyma (Fig. 34), while in the anterior proglottides they are somewhat larger, excepting in the interproglottidal region. Here they narrow down suddenly to a diameter of 8 μ . In the posterior unsegmented portion of the strobila they are quite flattened laterally, opposite the gravid uterine sacs (Fig. 19), on the whole somewhat smaller than in the jointed region and situated in the medulla but quite close to the longitudinal muscles (Fig. 18). The nerve-ring gives off besides the two chief nerve strands, eight others, four being grouped around each of the former (Figs. 34 and 35). It was at first difficult to decide whether these were distinct strands or only the intersections of an extensive meshwork of nerves situated in the cortical parenchyma and thus comparable to the "plasmatische canal system" of Sommer and Landois ('72); however, with further search eight strands could be followed throughout the segmented portion of the strobila. The difficulty in following them is due to the fact that the nervous branches given off mostly centrally are quite as large as the strands themselves and that they anastomose freely with one another and with the chief strands which are, however, much more distinct. These collateral nervous tracts gradually disappear with the appendages posteriorly. Thus they are apparently developed in connection with the extra musculature of the latter. Since the Golgi method of impregnation was not used on any of the material for this study, the nerve-strands were seen to be made up of only a very fine fibrillar meshwork containing extremely minute granules and vacuoles.

EXCRETORY SYSTEM.

There are three excretory vessels coursing throughout the strobila, a large median one evidently the morphological equivalent of the dorsal pair of many Bothriocephalids and two much smaller, ventro-laterally situated, all being located in the medullary parenchyma ("Markschicht") between the chief nerve strands (Figs. 34, 35 and 37). Immediately behind the nerve-ring the median vessel expands to form a somewhat spherical vesicle from 25 to 40 μ in diameter, into which the lateral

vessels open without any change of diameter. The junction of the median vessel and the vesicle is, on the other hand, not abrupt but gradual or funnel-shaped. In the scolex all three vessels take a comparatively straight course, gradually narrowing until, as they enter the first proglottis, they are, median, 30μ , and lateral, 8μ in diameter; while in the anterior proglottides they take the form of irregular spirals, the coils of the lateral vessels following more or less those of the median, excepting as they pass the interproglottidal space where they narrow down and straighten out slightly. The comparatively small size and straighter course of the vessels in the scolex is doubtless due to the great development of the dorso-ventral bothrial muscles through which they pass.

Posteriorly their course is modified by the development of the reproductive ducts in the median line. This applies more particularly to the larger median vessel, since the other two, being situated ventrolaterally, are not much disturbed. Between the sets of reproductive ducts the median vessel lies in the median coronal plane, separating the testes into two lateral fields (Figs. 17 and 18), while the smaller vessels are situated below the testes but within the ring of vitelline follicles. As the former approaches the cirrus-sac it usually rises (it is somewhat depressed in Fig. 38) and passes dorsally to the right or left, along the uterus-sac and over the ovary and lateral portion of the generative space to the median line again. However, it frequently crosses from one side to the other dorsal to the anterior end of the developing uterus-sac or the space between it and the opening of the vagina, as shown in Fig. 17. But the greatest changes in the course of these vessels comes when the uterus becomes gorged with eggs. The smaller vessels then appear greatly flattened laterally, within the testes that appear in these sections, and not so distinctly towards the ventral surface (Fig. 19). No trace of the larger median vessel is to be seen along the middle of the uterus-sac excepting in younger stages where it is in the form of an almost obliterated tube situated dorso-laterally. Anteriorly and posteriorly, however, in several series this vessel apparently passed right into the uterine sac tangentially, the opening thus being closed with a flap-like valve. While this was very difficult to make out and was considered of only secondary importance, it was thought that perhaps the much distended condition of many of the uteri in the posterior end of the strobila, especially behind the region of closure of the temporary uterine opening (*vide infra*), might be due to fluid from the median vessel escaping into the uterine cavity by the absorption or rupture, during distension, of the two extremely thin walls between.

The relations of the excretory vessels in what has been called the end-proglottis are rather peculiar. The median vessel (Fig. 12) gradually

expands to form a vesicle, varying in diameter from 25 to 55 μ and is situated immediately within an invaginated portion of the cuticle into which it opens. The openings of the lateral vessels are very difficult to make out, since they seem to be quite closed in many cases. All stages between the condition shown in Fig. 12 and one (in small scolices, Fig. 6) in which all three vessels opened separately on the concave posterior surface of the strobila, were observed. Thus it would appear that this species bears out Leuckart's view that the relations between the posterior openings of the excretory system are developed after some part (in most cases an earlier proglottis) has been separated from the strobila. In fact Fig. 12 is quite suggestive in all of its parts of a simple contraction of the hinder end of the worm to form a cuticular invagination, all of the vessels formerly opening on the outside.

The flame-cell (Fig. 13) is quite typical in structure and closely resembles that of the genus *Proteocephalus* Weinland, which has been studied by the writer, in that the vestibulum in which the "ciliary flame" is located is provided with peculiar darkly-staining longitudinal thickenings which do not seem to be mentioned in the literature on the excretory system of the cestodes. Their significance is, of course, merely conjectural. The cell-body is usually not as distinct as that shown in the figure, since the cytoplasm is quite clear, but the nucleus and basal body, as well as the "flame", are very easily made out in sections. It was found impossible to trace with certainty the canaliculus from the flame-cell to any of the larger vessels or smaller canals mentioned below. The flame-cells, themselves, are few in number and arranged more or less radially close around the large vessels.

The structure of the latter is shown to a certain extent in Fig. 14. Although the wall is extremely thin, the following parts could be discerned with high magnifications: a thin cuticular layer, with a distinct basement membrane, lining the tube; outside of that a clear line in transverse sections and dotted in longitudinal sections, thus resembling a layer of extremely fine cuticular muscles; and farthest peripherally, a condensation of cytoplasm with nuclei slightly smaller than those of the parenchyma, but hard to distinguish from the myoblastic nuclei near at hand. The circular striations appear to be more protoplasmic than muscular in nature and in many places cannot be differentiated from the basement-membrane.

Foramina secundaria are to be found in the anterior proglottides, especially on the outside of the ring to which the appendages are united (Fig. 35). The openings, themselves, are very minute (vide supra, under the cuticula), but the course of the capillaries leading to them through the subcuticula and peripheral portions of the cortical parenchyma is

clearly defined by the contents being highly stained by counterstains such as Orange G. In spite of the readiness with which these capillaries can be followed through the subcuticula, it was found impossible to trace them far towards the centre of the strobila, much less to connect them with any of the main excretory vessels. In the cortical parenchyma, however, they seem to unite to form a quite compact plexus, the diameters of the tubes of which vary from 2 to 6 μ . In the foremost joints there are more foramina secundaria on the anterior portion of the proglottis than on the auricular ring; while very few are to be met with in the scolex.

GENERATIVE ORGANS.

There is a more or less definite point in the strobila, at or about the 15th proglottis, ahead of which the genital organs do not seem to develop and behind which in older strobilas they appear very quickly. For instance, in one strobila 96mm. long and containing 55 joints, only the beginnings of the vitelline follicles are to be seen in the 14th joint; more and a few testes in proglottis 15; no appearance, in sections, of the generative ducts in the median line in the 14th; a mass of nuclei around the median excretory vessels (from transparent preparations) in 15; and an uterus full of eggs in 16! One must look then to the younger strobilas in which the proglottides are yet immature to see the earliest stages in the development of the reproductive organs, especially of the ducts. Here, of course, the stages are more gradual.

The genital ducts develop from a long, more or less cylindrical anlage surrounding the posterior half or two-thirds of the median excretory vessel, as shown in Figs. 15 and 16, which are from transparencies of proglottides 16 and 17, respectively, of a young strobila. Soon after the earliest traces of it can be seen in transparent preparations, the anterior end enlarges to become later the anlage of the cirrus-sac and entrance to the vagina, while the posterior end gives rise to the ovaries and organs of the "generative space", including the "uterine tube". From the middle part arises the "uterine sac", vagina and vas deferens.

All of the ducts seem to develop lumina almost simultaneously; but the vagina and cirrus do not pierce the ventral wall of the proglottis until somewhat later. Even the uterine sac approaches the ventral surface at its posterior end in the early stages. During the necessarily brief study of the development of the genital ducts the writer was able to corroborate, in general, the finds of Young ('13) and Schaefer ('13) as to the manner of formation of the lumen and epithelium from the syncytial anlage. Further remarks on the possible fate of the epithelial nuclei during the formation of the cuticle in the distal portions of the ducts will be met with below in connection with the more detailed description of the

cirrus and vagina. The fact, however, that the epithelium of the genital ducts of this species seems to be almost entirely a syncytium, even in the mature proglottides, should have special emphasis at this point.

The cirrus and vagina open very close together (actually from 0.02 to 0.07 mm. apart) on the ventral surface, about two-fifths of the length of the proglottis from its anterior end, in that part of the strobila where the auricles define the extent of the joints and relatively much farther forward posteriorly where proglottidation is absent (Fig. 17). This latter is partially due to the developing uterus-sac pushing them farther forward. There is no genital sinus, although in some states of contraction a more or less well-defined depression into which the two ducts appear to open, much resembles one,—in fact in some proglottides the cirrus and vagina open into each other on the ventral surface. The opening of the uterus is to be found on the ventral surface also, just ahead of the posterior end of the uterus-sac.

All of the reproductive system is accommodated in the medullary parenchyma, and, excepting testes and vitelline glands which are situated peripherally, the latter immediately within the longitudinal muscles of the parenchyma, all parts are much elongated antero-posteriorly, an adaptation apparently to the narrowness of the strobila. The limbs of the ovary are even squeezed together, making the whole organ horseshoe-shaped.

The "generative space", that is, the space enclosed by the limbs of the ovary, is filled with the proximal ducts of the female system (Fig. 27).

MALE SYSTEM

In young proglottides, in which the uterus-sac is short and narrow, the testes are from 55 to 70 μ in diameter and almost spherical in shape (Fig. 18), while in those in which the uterus is gravid they are ellipsoidal and from 70 to 115 μ long. Opposite the distended uterine cavity and near the reproductive ducts behind and forward they are more or less flattened—in the former position, greatly flattened (Fig. 19).

Like the vitelline glands the testes are continuous from one proglottis to the next. They are separated into two lateral fields by the medially situated genital ducts but come together in front and behind these, in the interproglottidal regions, to form a layer, also divided by the median excretory vessel (Fig. 18). This layer, which is situated in the medullary parenchyma, is made up of as many as six testes, in cross-section, three on each side and all in the medial coronal plane of the body. No more than one testes at a time is seen on each side of the section through the gravid uterus-sac (Fig. 19). In mature joints it is difficult to say how many testes there are, but from the posterior edge of the ovary of one set of genital organs to that of the ovary of the next there are about 40 in each lateral field, or about 80 in all (Fig. 17).

Each testis is surrounded by a very thin membrane which is directly continuous with the wall of the vas efferens (Fig. 20), a point which is rather difficult to make out since the testes are packed closely together and the vasa efferentia anastomose freely between them. Numerous, even about ten, developing cytophores in various stages may be seen in the younger testes.

The anastomoses of the vasa efferentia are best seen in the vicinity of the posterior end of the vas deferens (Figs. 17, 21 and 22) and not so well, among the testes laterally and in the interproglottidal regions. Thus it is conceivable that sperms developed in testes situated in the regions between the sets of genital ducts may find their way to the vas deferens of the same proglottis or to that of the proglottis ahead or behind, as the case may be. This would be facilitated by the rupturing of the delicate walls of the testes, which alone separate them in ripe joints, to form larger and more accessible channels for the sperms. Many instances of such ruptures were seen in the serial sections studied. Sommer and Landois ('72) found that the testes in the anterior part of the proglottis of *Dibothriocephalus latus* (Linn.) passed their sperms to the vas deferens of the joint ahead, but these relations were not found in this species in spite of the otherwise general resemblance between the arrangement of the genital ducts of the two. The vasa efferentia, themselves, vary considerably in diameter and possess very thin walls in which scattered and flattened nuclei are situated, as observed by Lonnberg ('91) in *Bothriocephalus rugosus* (Batsch), (Figs. 20 and 21).

Just ahead of the uterine opening the vasa efferentia unite to form a rather indefinite sperm reservoir, directly continuous with the posterior end of the vas deferens (Figs. 17, 21 and 22) and thus resembling the similar structure of many *Bothriocephalids*. Its walls are intermediate, as to the structure of the epithelium, between those of the vasa efferentia and those of the vas deferens (Fig. 21). The anterior boundary of the sperm-cistern is marked by the position of the foremost of one to three separate vasa efferentia which join the vas deferens on that side towards which the latter is directed in development (*vide infra*); rarely do vasa efferentia empty into the vas deferens ahead of this short region.

While it was found impossible to determine the lengths of the spermatozoa in sections, it was noticed that their anterior ends were differentiated as quite long and narrow cylindrical heads, slightly larger in diameter than the rest of the sperm, evidently pointed at their anterior ends and graduated less abruptly towards the tail. These heads stain very densely with Heidenhain's Iron-Haematoxylin and are consequently quite easy to pick out, while the other parts are scarcely discernable in the masses to be seen in various portions of the male ducts.

The *vas deferens* passes forward from the sperm-reservoir almost in the median line and dorsal to the uterus-sac, taking many irregular coils in its course (Fig. 17). In older proglottides, however, owing to the relatively enormous distension of the latter, it is pushed to one side until all parts, excepting those close to the *vesicula seminalis*, may eventually become obliterated. It seems to be crowded more often to the right, doubtless because of its position in younger stages; at any rate, the anastomotic reservoir formed at its posterior end by the *vasa efferentia* lies more often to the left. Fig. 17 is an exception to this, as it is a dorsal view.

In ripe joints before it is pushed aside by the developing uterine cavity, the *vas deferens* is tubular in shape, from 11 to 14 μ in diameter at its anterior end where it joins the *vesicula seminalis*, 17 to 25 μ at its middle and 22 to 35 μ at its posterior expansion, the latter being the diameter of the sperm-cistern. Later when it becomes gorged with sperms and the walls are, in consequence, thinner, the diameter varies from 40 to 55 μ .

The wall of the *vas deferens* consists of a low epithelium in which, as in the sperm-reservoir, no cell boundaries can be made out, supported by a poorly-developed basement-membrane (Figs. 21, 23a and b). It is thus a syncytium. In older proglottides, where the *vas deferens* contains sperms, the epithelium is flattened out so that the nuclei appear here and there along the duct as thickenings in an otherwise thin membrane. In young, and, as yet, non-functioning *vasa efferentia* nuclei from the outer layer of the anlagen remain close to the basement-membrane, especially towards the *vesicula seminalis*, to form the myoblasts of scattered and fine circular muscles (Fig. 23).

The *vesicula seminalis*, which is morphologically an expansion of the *vas deferens*, is situated close to the dorsal body-wall, immediately behind the cirrus-pouch (Fig. 17). It is ovate to spherical in shape in mature proglottides, before it is flattened against the latter by the gravid uterus-sac, with the more pointed end directed anteriorly, while in younger (but ripe) joints it graduates less abruptly posteriorly, that is, it is more broadly spindle-shaped. The wall has the same structure as that of the *vas deferens*, excepting that the syncytial epithelium is so much thinned out, especially when the organ is filled with sperms, that the nuclei, which appear singly or in groups of two or three and surrounded by small amounts of clear cytoplasm, seem to be applied to the inside of the basement-membrane itself. Outside of the latter there are to be seen numerous fine muscle-fibres, with their myoblastic nuclei, coursing in general longitudinal and circular directions. These are similar in structure to those surrounding the *vas deferens*. On

account of the extremely small size of these fibres it was found impossible to determine whether they are arranged in one or more layers. The following are the averages of the measurements of four vesiculae seminales:—

Length.	Width.	Depth.
0.140 mm.	0.092 mm.	0.090 mm.

The vas-deferens narrows very abruptly again to a diameter of 15μ as it enters the postero-dorsal portion of the cirrus-sac (Fig. 17) to become the ductus ejaculatorius. This portion of the duct takes three of four turns in the dorsal third of the cirrus-pouch and then passes on as an enlargement, the second vesicula seminalis, occupying approximately the middle third of the pouch (Fig. 38). While the walls of the proximal portion of the ductus ejaculatorius quite closely resemble in structure those of the vas deferens behind the larger or posterior seminal vesicle, those of the distal vesicula seminalis are very thin, showing few nuclei closely applied to the basement-membrane. The diameter of the duct at this point is about 38μ . As the junction between the ductus ejaculatorius and the inner vesicula seminalis is approached the epithelium becomes broken up into numerous processes which, however, did not appear to be true cilia. As a matter of fact cilia do not seem to present in any part of the male reproductive ducts.

The third division of the vas deferens within the cirrus-pouch, the cirrus proper, usually commences at the posterior pole of the latter, courses forward and then backward again to pierce the wall of the pouch and open on the ventral surface of the proglottis at the point shown in Fig. 17. The diameter of the cirrus at the bend in its course (Fig. 38, c) is about 20μ ; it enlarges gradually to 30μ before opening to the outside.

This region of the male duct can be evaginated, presumably, as in most cestodes, to form a copulatory organ, yet in all the material at hand not a single case of everted cirrus was observed. Consequently, nothing can be offered, in regard to its function, apart from the suggestion that from the frequent approximation of the male and female genital-openings, noted above, self-fertilization may possibly occur in this species. The structure of the cirrus would at least indicate that after eversion it might become quite an efficient organ. Its wall (Fig. 24) is composed of an inner lining of cuticle thrown into folds of varying heights, supported by a basement membrane which can be distinguished as such only in young stages. Outside of the latter appear two sets of circular muscles (Fig. 24, cm), separated by a comparatively clear protoplasmic area which is traversed by the longitudinal and the retractor fibres (rmp) and numerous

processes from parenchymatous cells lying farther out. The circular muscles increase in number at the opening of the cirrus and form a distinct sphincter. In that portion of the cuticle next the lumen, that is, towards the functional outer surface of the organ, there are to be seen numerous highly-staining granules which seem to be the bases of fine bristle-like processes extending into the lumen. While the granules show very plainly in sections, the processes themselves are difficult to make out clearly in many cases. They are, however, probably homologous with the spines, hooks, etc., described for the cirri of other species.

Fig. 25 shows a somewhat younger stage in the development of the cirrus than that shown in Fig. 24, and is of interest in connection with the problem of the formation of the cuticle. Considerable attention was paid to detail in this figure in order to bring out the following points. It will be seen that four or five nuclei lie close to the cuticle, in fact against the basement-membrane, while others farther out appear to be connected with the cuticle, or at least with the syncytium of protoplasm immediately outside of it, by fine strands. Many of these peripherally situated nuclei belong to the myoblasts of the circular muscle-fibres, as indicated by the letters "cmc", and some of them to the few longitudinal fibres, but they, especially the former, are fairly easy to distinguish from the majority of the number which have the central protoplasmic connections. Young ('13) and Schaefer ('13), working with different species of cestodes, came to quite opposite conclusions regarding the fate of the epithelial nuclei during the formation of the cuticle in the distal portions of the vas deferens and of the vagina. Young asserts that the nuclei disintegrate in situ as the cuticle is being formed, while Schaefer observed what is doubtless the migration of the nuclei into the surrounding cytoplasm. The writer does not pretend to have gone into the matter at all exhaustively, but from the few observations he has made on the material studied it would appear that this species falls into line with Schaefer's discoveries. At any rate, no conclusive evidence of nuclei having disintegrated in situ in either the cuticle of the cirrus or that of the vagina was met with, but appearances like that shown in Fig. 25, where the original syncytial nuclei seem to have migrated some distance from the developing cuticle, retaining their protoplasmic connections and possibly functioning in the formation of that layer by secretion, are very common. In later stages, evidently when the cuticle is completely formed, these connecting strands fuse with the general mass of parenchymatous cytoplasm surrounding the cirrus and its retractor muscles, giving the appearances shown in Fig. 24. More will be given below in this connection under the vagina which, on account of its comparatively greater length, is better adapted to show the stages in the development of the cuticle.

The cirrus-sac is situated about midway between the dorsal and ventral surfaces of the proglottis, immediately ahead of the vesicula seminalis (Figs. 17 and 38). In shape it is spheroidal, being flattened laterally and somewhat protracted ventro-posteriorly where it follows the cirrus to the latter's opening, as the following measurements indicate: longitudinal diameter, 0.16 to 0.21 mm.; transverse diameter, 0.14 to 0.16 mm.; vertical diameter, 0.18 to 0.20 mm.

The wall of the cirrus-pouch, although quite thin, is wholly muscular and composed of two sets of fibers which can be better distinguished as such in younger proglottides than in older or gravid joints where they course irregularly and obliquely. Of these two sets the inner are circularly disposed while the outer are arranged longitudinally, thus corresponding to the description, by Sommer and Landois, of the parts in *Dibothriocephalus latus*. The fibers in the postero-dorsal portion of the wall intermingle with those of the vesicula seminalis; postero-ventrally they converge towards the opening of the cirrus, around which, with the dorsoventral parenchymal fibers of the immediate neighbourhood, they attach to the cuticle of the ventral surface. A very few fibers, on the other hand, difficult to distinguish from these dorsoventral parenchymal muscles, pass from the dorsal wall of the cirrus to the dorsal body-wall. Thus retraction of the cirrus, if, indeed, it is ever everted, would appear to be brought about by the mere elasticity of its tissues and of those surrounding it.

The dorsal half to two-thirds of the space within the cirrus-sac, which accommodates the ductus ejaculatorius and its expansion, the second vesicula seminalis, is filled with numerous parenchymal cells grouped irregularly around the duct outside of the fine longitudinal muscular fibers following the course of the latter. The myoblastic nuclei of these are visible as spindle-shaped, highly-staining bodies, closely applied to the fibers themselves. The ventral half to one-third of the space, on the other hand, appears much more compact in sections and transparent preparations, since it is in this region that the large retractor fibers of the cirrus are located. The latter are arranged in groups (Fig. 24) and attached evidently to the cuticle centrally, while they intermingle peripherally with the fibers composing the wall of the sac. The myoblastic nuclei are related to these fibres as in the case of the longitudinal muscles of the parenchyma, that is, one nucleus is associated with three or four fibrils. In addition to the circular fibers situated immediately outside of the cuticle of the cirrus proper, there are other finer ones to be seen for some distance beyond the cytoplasmic area, above-mentioned, intermingling with the large retractor fibers (Fig. 24).

FEMALE SYSTEM.

The vagina opens on the ventral surface of the proglottis immediately behind the opening of the cirrus and from 0.02 to 0.07 mm., from it (Fig. 17). While in most cases the aperture is circular in outline and from 20 to 30 μ in diameter, it is occasionally found in preserved material to be transversely elongated, more especially when it approximates the male opening (vide supra). The first portion of the vagina is in the form of a somewhat elongated vesicle, 56 μ in transverse diameter and situated beneath the vesicula seminalis; it is quite comparable, in shape at least, to the "Scheideneingang" of Sommer and Landois. After being slightly deflected dorsally, as in *D. latus*, the duct then passes back along the ventral side of the uterus-sac, on either side of the median line, or crosses from one side to the other at different levels ahead of the uterus-opening—in young proglottides ahead, necessarily, of the limb of the uterus directed towards the latter. In either case it turns to the median line again close to the posterior wall of the uterus-sac, and then passes over the ovarian isthmus and into the "generative space" where it expands, as it courses ventrally again, to form a receptaculum seminis.

The structure of the vagina is quite comparable, on the whole, to that of the vas deferens. Posteriorly it is lined with a syncitial epithelium, supported by an indistinct basement membrane which is relatively somewhat thinner than that of the vas deferens of the same proglottis, excepting in the region of the receptaculum seminis (vide infra). This is doubtless due to the fact that during the period of differentiation of the two tubes from the middle and narrower portion of the common anlage of the genital ducts, the vagina is somewhat in advance of the vas deferens, that is, it develops a lumen slightly previous to the formation of one in the latter, and then, evidently keeps in advance of it during subsequent growth and distension. From a point opposite the anterior end of the uterus-sac to its opening the vagina is lined with a cuticle which in many cases is lacerated and torn, especially at the surface next to the lumen. In this region, at the proper stage, that is, about the time when only a few eggs appear in the uterus-sac, what was considered by the writer to be the transformation of the epithelium into the cuticle can be observed much more clearly than in the case of the cirrus where only a comparatively short length of duct develops a cuticle. This seems to be brought about almost wholly by the sinking of the nuclei into the surrounding mass of cells derived from the outer layers of the anlage and lying outside of the basal membrane and circular muscles, and by the subsequent alteration of the epithelial substance to form the homogeneous cuticle. Very few nuclei in their passage through the membrane were seen, since no lengthy study of this subject was undertaken and since, as suggested

by Schaefer the process takes place, in all probability, quite rapidly, thus rendering the finding of the nuclei in all of the stages a matter of some difficulty in a comparatively small number of series. Three figures are given, however, to illustrate what was observed by the writer in this connection. Fig. 39 is a photograph of a coronal section through the first portion of the vagina, the entrance to the vagina being shown at "v". The latter is seen to be surrounded by a number of radiating cylindrical cells with rounded peripheral ends towards the parenchyma, somewhat resembling the cells of the subcuticular layer. They are much more numerous around the enlarged portion of the vagina than around the duct farther back. At "x" one of these elongated cells, with the nucleus situated at its extremity, is attached to the cuticle tangentially and in such an intimate manner as to lead one to think that it still functions, possibly, in the formation of the latter. Again at "x" and "y", Fig. 40, two nuclei with the surrounding cytoplasm appear to have passed through the basement-membrane but to have gone only a short distance beyond it. A similar case is shown in Fig. 41 at "y", while at the point marked "x" a nucleus half way through the basement-membrane is to be seen. As the nuclei pass through the latter they are surrounded in many cases by clear areas, possibly cytoplasm quite thin in consistency, as noted by Schaefer in *Bothridium pythonis* Blain. Thus it appears—to the writer, at least—that, in the transformation of the epithelium of the distal portions of the vagina and vas deferens into the cuticle, the nuclei of the former pass into the surrounding parenchymatous tissue, and may there function in the formation of the latter. While the above evidence is scarcely to be considered as conclusive, it is given in the hopes that it will be at least suggestive to the reader in connection with the question of the formation of the cuticle in cestodes, which is again occupying the attention of helminthologists.

The musculature of the vagina is composed of circular fibers only, as in *Cyathocephalus truncatus* (Pallas), which are situated immediately outside of the basement-membrane. Very few of them surround the greater part of the canal, including its anterior enlargement, but a comparatively large number are developed in the short region between the latter and the opening to form a powerful sphincter, 30 to 40 μ in length.

From a point opposite the posterior end of the uterus-sac and ventral to the uterine tube ("Uteringang") the vagina gradually enlarges as it passes dorsally over the ovarian isthmus to form the *receptaculum seminis*. The posterior, rounded end of the latter is situated within the generative space dorsal to the oocapt, with its longitudinal axis almost vertical (Fig. 27). The diameter of the tube at this point varies from

30 to 45 μ , depending on the amount of its distension with sperms. The receptaculum seminis is lined with a direct continuation of the syncytial epithelium of the vagina, in which, however, some tendency to form cell-boundaries appears, especially in the earliest stages. No valve-like modifications of the wall, as described by some authors for this part of the vagina of other species, were seen; there is simply a gradual enlargement of the duct up to the sudden constriction about to be mentioned. Furthermore, although the epithelium of the vagina and receptaculum seminis shows in many cases fine processes of different sizes, directed towards the lumen, these were not considered to be cilia, since, in the same regions of the other proglottides of the same chain, the epithelium was quite smooth and bounded by a more or less distinct membrane. There are few circular muscle-fibers surrounding the receptaculum seminis until a point is reached, immediately ahead of the constriction which bounds it proximally. Here they are greatly augmented and directly continuous with a well-developed musculature which surrounds the beginning of the spermaduct (Fig. 26). This musculature is evidently developed for the purpose of passing along, by swallowing movements, only a few sperms at a time, as indicated in the drawing which shows a string of sperms connecting the mass in the center of the receptaculum with the spermaduct. The latter in all such cases is filled with spermatozoa.

Immediately behind and ventral to the receptaculum seminis the vagina narrows down abruptly to form the spermaduct. While its first portion, as indicated in Fig. 26, is very small, being only from 5 to 10 μ in diameter, it soon enlarges to almost twice that diameter, the size which obtains throughout the rest of its course. On account of the intense staining powers of the surrounding musculature it is very difficult to ascertain the nature of the wall at this level; however, it is composed of a very thin epithelium in which no nuclei were seen. On the other hand, certain nuclei situated outside of the basement-membrane and connected with it by cytoplasmic strands, on the whole reminding one of the radiating cells surrounding the cirrus and the entrance to the vagina, may possibly have been located within the epithelium at an early stage in development. Some of them are obviously the myoblastic nuclei of the circular muscles. To determine the exact origin of these nuclei it would be necessary to make a special study of the development of the ducts of the generative space, since the musculature of the spermaduct arises very early, even before some of the other ducts in the immediate neighbourhood are completely differentiated. The circular fibers diminish in number throughout the remainder of the duct, but are much more numerous than the few longitudinal fibers, arranged somewhat spirally outside of them.

The ovary is an annular or closed horseshoe-shaped organ, situated ventrally at the posterior end of the middle field of the proglottis (Fig. 17). Although in most cases it appears to be completely closed posteriorly, it is in reality made up of two limbs,—they can be distinguished as such in the very young stages of development—connected anteriorly by an isthmus, on the ventral side of which is situated the oocapt. The limbs themselves are generally enlarged anteriorly, so that, on the whole, they somewhat resemble those of *D. latus*, which are, however, widely separated behind (Sommer and Landois). The organ is surrounded by a very thin wall and is divided by a continuation of the same into a number of irregular, tubular compartments which accommodate the ova. Scattered throughout these partitions and the outer capsule itself, very small, flattened nuclei, from 1 to 2 μ in diameter, are to be seen. This description applies to all of the ovary, excepting that portion of the isthmus lying quite near the oocapt. Thus, from the fact that the isthmus—with the contained ova—is solid, it would appear that the views of Sommer and Landois and not those of Leuckart, who considered the isthmus or "bridge" to be a mere duct-like portion of the organ for the passage of the ova, are applicable to this form.

The largest ova (Fig. 28a), which appear in the ventral part of the isthmus and are thus ready to be passed on for fertilization by the oocapt, vary in longitudinal diameter, since the cytoplasmic outline is somewhat irregularly oval, from 10 to 12 μ . The greater part of the comparatively large nucleus, which is about 7 μ in diameter, stains much less deeply with Heidenhain's Iron-Haematoxylin than does the surrounding protoplasm; the "nucleolus", on the other hand, comes out extremely dark blue. With Mallory's stain, however, it appears orange, which colour is seen in no other part of the body. Consequently, the nucleolus seems, from its staining powers, to be a definitely functioning body and not a mere aggregation of nucleoplasmic particles. Yet such aggregations, quite as large as the nucleolus itself, are to be seen in other parts of the nucleus; so that from this and the further fact that the outline of the nucleolus is very often irregular, it is a matter of conjecture as to what is the true nature of the body in question. In the cytoplasm of many ova small clear areas, often provided with darkly staining bodies resembling nucleoli, are to be seen (Fig. 28b and c). Some of these may be nuclei forming in the protoplasm *de novo* (after Young's views), but others so closely resemble small free ova as to lead one to think that they may be abortive ova which have come into intimate contact with the cytoplasm of the normal ova and been subsequently absorbed by them, stages in the process of which absorption are probably represented in Fig. 28c.

The oviduct begins on the ventral surface of the ovarian isthmus with the oocapt which is a broad funnel-shaped or hemispherical structure directed ventrally in the median line (Fig. 27). The diameter of the latter, using the outer limits of the circular musculature as the boundary, since the organ is very gradually continuous with the wall of the isthmus, varies from 15 to 25 μ . It is lined with a cuticle-like substance, which shows no nuclei for a short distance, and is surrounded by a system of circular muscles, arranged and extended quite like those of the spermaduct and posterior end of the receptaculum seminis. Furthermore, the resemblance in structure is the more exact from the fact that the constricted portion of the duct, which immediately follows the muscular funnel, is surrounded by radially arranged nuclei, many of which belong, of course, to the myoblasts of the circular fibers. The constricted part has a diameter of from 8 to 9 μ ; after which the oviduct gradually enlarges to 15 μ , as it courses to the right or left and posteriorly until it meets the spermaduct almost in the median line of the proglottis. The wall is made up of a ciliated epithelium, in which are to be seen one layer of nuclei but no distinct cell-boundaries in the somewhat vacuolated cytoplasm, supported by a well-developed basement-membrane.

A short distance from its union with the spermaduct the oviduct is joined by the short duct from the yolk-reservoir. Just behind this point there is a slight constriction, around which the circular muscles are augmented in number to form a small sphincter while they are accompanied by a few longitudinal fibers.

Two vitelline ducts, each about 6 μ in diameter, collect yolk from the lateral fields of vitelline follicles and pass towards the median line to unite either within or outside of the generative space ventral to the ovary. Union within the latter is the usual arrangement, in which case each duct is accommodated in the groove situated on the ventral surface of the ovary between the oocapt and the anterior end of the limb on each side. Each of these ducts may receive material from a few follicles on the opposite side of the proglottis, but, in general, it collects from the same side to which it is directed. Their walls are composed of a thin epithelium, showing small flattened nuclei distributed at wide intervals, on the whole resembling those of the vasa efferentia. Their courses are easily followed by observing the, in many places, greatly extended yolk-cells on their way to the yolk-reservoir (Fig. 29b.). On the other hand, the arrangement and structure of the smallest ducts in immediate connection with the yolk-follicles were not determined to the writer's satisfaction, since the latter are packed so closely together; but from various appearances they seem to anastomose.

The common duct, which is quite short (Fig. 27), is slightly larger than the collecting ducts, and its epithelium contains relatively more nuclei. It is furthermore provided with cilia, directed towards the yolk-reservoir.

After passing for a short distance dorsally and towards either side of the proglottis, depending on the arrangement of all of the ducts in the generative space, this common yolk-duct expands into the vitelline reservoir, an ellipsoidal or somewhat spherical sac varying from 25 to 55 μ in diameter according to the amount of yolk it contains. Even when yolk is absent, however, it is larger than the common duct and shows very few cilia; thus it seems to be a true reservoir, in that it is possibly differentiated early in development, and not a mere temporarily functional dilatation. The epithelium is naturally considerably distended and flattened by the contained yolk. The reservoir unites with the oviduct through a short length of common vitelline duct whose structure is identical with that of the above-mentioned portion.

The vitelline follicles, like the testes, are situated in the medullary parenchyma, that is, within the longitudinal muscles and consequently within the nerve-strands (vide supra), thus resembling, as to situation, those of the genera, *Ancistrocephalus* Montic., and *Anonchocephalus* Luehe, (Luehe, '02). There they form a continuous cylinder from one proglottis to the next, enclosing the excretory ducts and reproductive organs, including the testes, but broken ventrally and dorsally by middle fields corresponding in extent to the region occupied by the generative ducts, that is, from the anterior end of the cirrus-pouch to the posterior end of the ovary (Figs. 17, 18 and 19). Posteriorly they crowd the ovarian limbs very closely, a few even passing above and below their hinder ends. Thus, in extent, the vitelline follicles are comparable to those of *Cyathocephalus* (Kraemer '92), *Schistocephalus* (Kiessling '82), and *Bothriocephalus dendriticus* Nitzsch and *B. ditremus* Crep. (Matz '92), excepting that in the three latter forms the dorsal middle-fields are occupied by them, while in the first genus neither dorsal nor ventral middle-fields are left free of follicles. In *D. latus*, on the other hand, both fields accommodate no vitelline follicles (S. and L. '72).

The follicles themselves are usually spherical to ellipsoidal in shape; but in ripe joints, where they are very closely packed together, the outline is somewhat polyhedric. Furthermore, they vary greatly in size, the smallest being only about 8 μ in diameter, while the largest, which are more numerous, are even 50 μ . The yolk-cells also vary in size, being from 5 to 15 μ in length, obviously owing to their relative states of maturity. This is shown in Fig. 29a, which also gives some idea of their variety of outline. The latter, however, seems to be the result of the accommodation of a number of semi-fluid bodies within a fairly tense

membrane—the yolk-cells within the follicular wall. That the yolk-cells are semi-fluid in consistency cannot be doubted when one observes them in their passage through the vitelline ducts, as noted above in connection with the description of the latter, and as shown in Fig. 29b, where the nucleus with its surrounding clear area is distending the wall of the duct. The nucleus and, for that matter, the whole cell in many cases, resembles that of the ova; in fact, it is often quite difficult to decide which is the ovum in the egg-complexes to be found in the uterine tube. In most follicles the smaller cells are arranged around the wall more or less like an epithelium, as described by Sommer and Landois for *D. latus*, while the larger ones are to be found in the middle. The wall itself is a very thin membrane in which no definite nuclei were seen, although small flattened nuclei situated between the yolk-cells and close to the wall may belong to it. Perhaps the most noteworthy peculiarity of the yolk-cell is the large almost clear area to be seen in the cytoplasm, often surrounding the nucleus (Fig. 29), which is doubtless the fluid yolk which will later be absorbed by the developing egg.

A short distance from the point where the oviduct receives the common vitelline duct are located the shell-glands. Here the oviduct expands slightly—to a diameter of 20μ . In most of the series examined the shell-glands formed a sort of vacuolated meshwork, in which, although there were to be seen well-developed nuclei, 4 to 5μ in diameter, it was extremely difficult to distinguish individual glands. However, in one series, where quite a length of oviduct was cut longitudinally, two or three club-shaped unicellular glands could be made out (Fig. 30). Their connections with the former were in the form of darkly-staining bars traversing the epithelium between lighter areas of about the same widths. Furthermore, numerous thread-like processes situated in the lumen, of the oviduct and directed towards the uterine tube corresponded with these dark bands, at least in position, since they were divided into groups, each group being opposite a dark band, as shown in the figure. While the outlines of the glands are quite difficult to discern, their connections with the oviduct are readily seen in sections through the region in almost any plane, tangential sections, for instance, showing dark circular spots on a much lighter background. Again, in younger proglottides treated with Mallory's stain, the glands and the otherwise dark bands appeared much lighter than the epithelium, which fact further supports the view that they are related anatomically. Thus, from the foregoing description, it appears that the processes in the lumen probably constitute the material secreted by the glands, and that this material is passed along from the bodies of the cells through the narrow necks which act as duct-lets, suggestions which are strengthened by the facts that the so-called

secretion is to be seen mostly opposite the glands and that the ductlets have very thin but distinct walls.

The uterine tube ("Uteringang") is usually considered to commence immediately beyond the shell-glands, but in this species its first portion so closely resembles the oviduct posterior to the latter that the writer is inclined to place the region of demarcation somewhat farther ahead. The circular muscles are better and more uniformly developed, but what appears to be a decided augmentation in the number of cilia is probably a continuation of the threads of material secreted by the shell-glands. While the shell-gland region of the tube is directed dorsally, anteriorly and generally to the right of the proglottis, the beginning of the uterine tube makes a sharp turn and then passes backward again or expands immediately into what might be called the second division of the uterus, (Fig. 27). But this is not the second division of the uterus according to Braun ('00), since he does not seem to recognize in the "Uteringang", or uterine duct, two divisions, differing histologically; his second division is the uterus-sac, or "Uterushöhle". In this species it is in the form of a tube from 25 to 55 μ in diameter, the walls of which are very thin and composed of a greatly extended epithelium in which quite flattened nuclei appear at irregular intervals. Commencing in the dorsal portion of the generative space, it courses forward in the median line above the ovarian isthmus as a somewhat flattened spiral, and in ripe proglottides often narrows down appreciably before entering the uterus-sac tangentially. It is usually filled with young eggs, each composed of many yolk-cells surrounding the "egg" (fertilized ovum) or a small number of cells resulting from the first divisions, all enveloped by a thin shell. In development the uterine duct is quite similar to the other ducts, possessing in the earliest stages a syncitial epithelium developed from an axial strand of cells surrounded by another layer of several cells in thickness, between which the basement membrane appears.

The uterus-sac ("Uterushöhle") arises in the same way from the middle piece of the elongated anlage (Figs. 15 and 16), but is from the outset distinctly separate from the uterine tube, the latter opening into it dorsally and slightly ahead of its posterior end. Beneath this opening the uterus-sac sends a diverticulum ventrally to the point where the aperture will later appear, while the remainder of the organ is directed forward in the median line some distance from the ventral surface, at first as a narrow tube, later as an elongated sac (Fig. 10). At this stage the wall of the uterus resembles, in general, that of the generative ducts. It is composed of a syncitial epithelium with scattered nuclei, a well-defined basement membrane, and outside of the latter a thin layer of parenchymatous cells. All of these parts are thinned out considerably

with growth, so that eventually, in gravid joints, the wall appears as a very thin membrane, showing practically no structure (Fig. 19). In slightly younger stages than that shown in Fig. 17 the lumen is not so uniform in outline, since its anterior half is divided into shallow evaginations, somewhat comparable to those seen in the uteri of the species of the order *Tetraphyllidea*. These soon become obliterated, however, the inside of the wall in gravid conditions begin quite smooth.

The opening of the uterus-sac, situated, as shown in Fig. 10, towards its posterior end, seems to function for a short time only, since in the longest strobilas no trace of it was found near the end of the chain. In the middle region, on the other hand, it appears as a very narrow slit, about 0.1 mm. in length, in only a few proglottides. Furthermore the uteri in which these openings are to be seen are generally almost free of eggs, as if the openings had been used for the dispersal of the eggs in the usual manner among the Bothriocephalids, while those behind the region in question are tensely filled. These facts would lead one to think that in most proglottides the eggs are freed by the rupture of the uterus and the body-wall, as in the higher cestodes, beginning with the *Tetraphyllidea*, while the uterine aperture either functions for a short time only or in proglottides, probably more or less constant in number and location. Fig. 31a is a view of the opening drawn from a transparent preparation in the uterus of which were comparatively few eggs. It is seen that the slit is surrounded by a clear area beyond which there is a more deeply-staining region. The latter is in reality made up of radially arranged nuclei which are related to the clear area in a manner better shown in coronal sections of stages prior to the breaking through of the slit (Fig. 31b). Here they are seen to be connected with the dark line, where the slit will appear later, by fine striations which continue farther out into the surrounding cytoplasm. Whether these radiating nuclei form a glandular organ around the aperture or give evidence of a migration from the clear area which remains as a cuticular rim, is difficult to say; but, from the close resemblance to the structure of the cirrus and of the entrance to the vagina, the writer is inclined to the latter view.

Development of the fertilized ovum, which begins, as mentioned above, in the uterine duct proceeds in the uterus-sac, eggs, bearing oncospheres, being obtainable from proglottides situated towards the posterior end of the strobila.

The egg of this species is an ellipsoidal structure, from 60 to 70 μ in length and from 40 to 43 μ in breadth. The shell is uncolored and perfectly transparent, so that the contents can be observed quite easily (Fig. 32). It is lined by a very delicate membrane which, however, can be seen only when it is, in some cases, separated from the former (Fig. 33).

The embryo is composed of two portions, an inner, the oncosphere, and an outer, the mantle or so-called ectoderm, well supplied with cilia. The movements of the latter can be seen even when the embryo is within the shell, especially if a little pressure be applied to the cover-glass. In this case they vibrate so vigorously that the whole embryo is driven to the larger end of the egg, and numerous, supposedly vitelline granules, are kept continually in motion, and, at the same time, arranged in two groups, one close to the mantle and, to all appearances, among the bases of the cilia, and the other in the smaller posterior end of the egg. The shell is provided at its anterior end with a well-defined operculum, the raising of which, evidently due to the pressure from within, permits the escape of the embryo. This, however, is a somewhat difficult matter on account of the size of the latter, as can be seen from Fig. 33. As fast as the cilia are freed they proceed to vibrate strongly in the surrounding saline solution, and as soon as the embryo has escaped from the shell it swims away quickly, taking either straight courses or moving about erratically in irregular curves. It was also noticed that the cilia are all directed posteriorly from what might be called the apex of the body, both within and without the shell,—posteriorly, since this apex is anterior, not only from its direction during motion, but from its being situated at the end of the oncosphere opposite to that which accommodates the hooks. While the mantle is comparatively constant in size, its diameter being about 45μ , the oncosphere varies from 30 to 35μ in length. Practically no structure was observed in the substance of the mantle itself. The oncosphere, on the other hand, shows the usual three pairs of hooks, a pair of flame-cells and a few spherical bodies of doubtful significance (Fig. 32). The movements of the hooks and of the body of the oncosphere are quite typical. They take place even when the embryo is yet within the shell, but, as has been verified in many preparations, only when the whole egg has been stimulated by pressure, in which case they are quite irregular and necessarily considerably restricted. They are perhaps a little freer when the embryo, including the ciliated mantle in situ, is liberated. At rest the three pairs of hooks are arranged, as shown in Fig. 32, in the form of a tetrahedron, the apex of which is situated at the center of the oncosphere while the base is directed posteriorly. From this position the peripheral ends of the hooks approach each other until they are quite close together, while the central ends diverge towards the bounding membrane of the oncosphere. This causes a slight retraction of the tip of the latter. Then follows a comparatively vigorous separation of the hooks, to the extent that their outer ends are about 180° apart while the inner are close together. At the same time the individual hook protrudes from the surface of the

oncosphere up to the small process, situated a short distance from its tip (Fig. 32). This process and the slightly swollen central end of the hook seem to act as bases of attachment for what appears to be a well-developed musculature actuating them. The hooks again approach and the whole cycle is completed. In the most vigorous specimens these movements take place at the rate of about three per minute. As might be expected, the slightly smaller anterior end is much affected by the movements of the other end; however, it exhibits movements of its own, consisting of small waves of contraction commencing at the inner ends of the hooks and passing forward, thus in a direction opposite to those seen in the plerocercoid and in the young strobila.

Concerning the life-history of this species nothing can be offered at the present. It was only noticed, as mentioned at the outset, that plerocercoids a few millimeters in length were found in the intestine of the host along with the largest strobila taken. The food of *Amia calva* consists, however, evidently entirely of small fish, mostly minnows, and it is possible that one or more species of these are the intermediate hosts.

SUMMARY.

The form of the body of this worm is peculiar in that proglottidation is expressed externally only in the anterior end of the strobila, beginning immediately behind the scolex. Here the proglottis is provided at its hinder end with four ear-like appendages directed posteriorly, which, in conjunction with their fellows of the neighbouring joints, may act as important accessory organs of attachment, perhaps by forming temporary suckers or using certain rows of spines, arranged around their edges, to obtain a hold on the mucous membrane of the host's intestine. Posteriorly these appendages disappear, leaving no indication of proglottides apart from the sets of reproductive organs which follow each other at regular intervals in the usual manner.

The scolex differs little internally as well as externally from the foremost joints, the two bothria or suckers being comparatively feebly developed.

The musculature is particularly well expressed in the jointed region of the strobila which is consequently the most mobile. All of the usual groups of muscles to be seen in Bothriocephalids are present, the external longitudinal fibers being quite distinct from the inner or longitudinal muscles of the parenchyma but confined to the anterior end only of the strobila, while the outer transverse series is divided into two sets on each surface of the proglottis, the fibers of which are directed postero-laterally and thus made to decussate in the mid-line. The individual fibers of nearly all of the groups of muscles are characterized by having their

cortical or contractile layers divided up into a number of fibrils, which, however, still retain their connections with the protoplasmic substance of the myoblasts.

The nervous system consists of two chief strands, situated laterally in the medullary parenchyma ("Markschicht") and united beneath the tip of the scolex to form a very small ganglionic ring. Connected with these are eight collateral strands, four located around each chief strand, which appear in the jointed portion of the strobila only.

The excretory system is composed of one large median vessel,—the equivalent of the usual dorsal pair—and two smaller, situated laterally and ventrally. All of these unite in the scolex to form a median vesicle accommodated in the hollow behind the nerve-ring. Foramina *secundaria* and flame-cells are fairly numerous, but their connections are difficult to trace.

The genital organs are simple, on the whole resembling those of *Dibothriocephalus latus* (Linn.). The genital apertures are all situated on the ventral surface in the median line, that of the vagina close behind the cirrus-opening towards the anterior end of the proglottis, that of the uterus much farther back and evidently a temporary aperture only. There is no distinct genital atrium or cloaca.

The testes are all in one plane and separated into two lateral fields by the median excretory vessel. Opposite the genital ducts both testes and vitelline glands separate dorsally and ventrally to leave clear "middle fields". The vas-deferens, which courses in the median line dorsal to the uterus-sac, is provided, at its posterior end near the middle of the proglottis, with a sperm-reservoir, and with a large almost spherical seminal vesicle situated immediately behind the cirrus-pouch. The latter is spheroidal in shape, simple in structure, and contains the continuation of the vas-deferens, divided into three regions, an ejaculatory duct, a second seminal vesicle and the cirrus. The cirrus is lined with cuticle in which there are small stout spines and around which there is a series of well developed circular muscles.

The vagina, the entrance to which is also lined with cuticle and supplied with a sphincter muscle, courses ventrally and expands within the "generative space" to form a seminal receptacle, sharply separated from the very small and short continuation, the spermaduct which unites with the oviduct in the usual way. The ovary and shell-glands are median and respectively ventral and dorsal. The yolk-glands are composed of numerous follicles, arranged cylindrically around the testes,—both within the longitudinal muscles of the parenchyma. There is a large yolk-reservoir, situated in the generative space. The uterus is divided into two distinct portions from the earliest appearances in the

common genital anlage, a much-coiled, proximal, thin-walled tube, the uterine tube ("Uteringang"), and a capacious uterus-sac ("Uterushöhle") which, when gravid, occupies almost the whole of the central portion of the proglottis. The eggs are provided with opercula.

All of the genital ducts are lined with an epithelium which, on account of cell-boundaries being almost entirely absent, is of the nature of a syncytium. In certain regions, namely, in the cirrus and in the entrance to the vagina, this syncytial epithelium becomes transformed into cuticle with an accompanying migration of its nuclei through the basement-membrane and into the surrounding parenchymal cytoplasm.

From the foregoing description it is to be seen that, although this species is in most respects a typical Bothriocephalid, its characters are such as to render the placing of it in the existing classification of the families and genera of the order, Pseudophyllidea, a matter of considerable difficulty. However, since this subject is dealt with in another paper, as mentioned at the outset, it will be sufficient to state here that, so far as the writer has been able to ascertain, this is a new species of cestode which must also be accommodated in a new genus. Consequently, the following names are proposed: Genus, *Haplobothrium* (ἁπλοῦς, simple; βόθριον, a small hollow or trench); species, *globuliforme*, (*globulus*, a bead; *forma*, shape or form), the significance of which specific name has been referred to above.

The type-specimen of this species is included in the writer's private collection, while a co-type has been donated to Dr. H. B. Ward of the University of Illinois.

Biological Department,
University of Toronto,
August, 1914.

EXPLANATION OF FIGURES.

All of the figures are camera-lucida drawings, excepting Figs. 34 to 41, inclusive, which are photomicrographs of sections or portions of sections.

<i>c</i> , cirrus.	<i>olm</i> , outer longitudinal muscles.
<i>ccm</i> , circular cuticular muscles.	<i>pc</i> , cells of parenchyma.
<i>cm</i> , coronal muscles.	<i>rmp</i> , retractor muscles of cirrus.
<i>cmc</i> , circular muscles of cirrus.	<i>rs</i> , receptaculum seminis.
<i>cn</i> , collateral nerves.	<i>rvd</i> , right vitelline duct.
<i>cu</i> , cuticula.	<i>sc</i> , cells of subcuticula.
<i>cub</i> , cuticula of cirrus.	<i>sg</i> , shell-gland.
<i>D</i> , dorsal.	<i>sr</i> , sperm-reservoir.
<i>g</i> , ganglionic ring.	<i>t</i> , testes.
<i>ivs</i> , second vesicula seminalis.	<i>us</i> , uterus-sac.
<i>lev</i> , lateral excretory vessel.	<i>ut</i> , uterine tube.
<i>lm</i> , longitudinal muscles of parenchyma.	<i>V</i> , ventral.
<i>mev</i> , median excretory vessel.	<i>v</i> , vagina.
<i>n</i> , nucleus.	<i>ve</i> , vas efferens.
	<i>vf</i> , vitelline follicles.
	<i>ns</i> , nerve strand.

PLATE V.

- Fig. 1. Scolex and first three proglottides, $\times 32$.
 Fig. 2. 12th, 13th and 14th proglottides, $\times 8$.
 Fig. 3. Proglottides 20 to 25, inclusive, coronal view, $\times 8$.
 Fig. 4. Same, lateral view, showing disappearance of auricular appendages, $\times 8$.
 Fig. 5. Young "scolex," showing beginning of proglottidation, $\times 16$.
 Fig. 6. Smallest plerocercoid observed, $\times 16$.
 Fig. 7. Longitudinal section through the cuticle and subcuticle: *cu'*, outer and *cu''*, inner layers of the cuticle; *fs*, foramen secundarium of the excretory system; *bm*, basement membrane; *lcm*, longitudinal cuticular muscles, $\times 1600$.
 Fig. 8. Longitudinal section through the tip of an appendage, showing the minute spines, $\times 1500$.
 Fig. 9. Relations between myoblasts and muscle-fibrils: a, coronal fiber; b, c and d, myoblasts of longitudinal muscles of the parenchyma, $\times 1500$.
 Fig. 10. Transverse sections of muscle-fibers: a and b, from the external longitudinal series of the parenchyma; c, from the dorso-ventral group, actuating the bothria; f, fibrils, $\times 1500$.

- Fig. 11. Reconstruction of the nervous system in the scolex and anterior proglottides, $\times 40$.
- Fig. 12. Coronal section of the "end-proglottis", showing the relations of excretory vessels; *cv*, dilatation of the median excretory vessel, $\times 150$.
- Fig. 13. Flame-cell: *bb*, basal body; *cf*, ciliary flame, $\times 300$.
- Fig. 14. Transverse section of a lateral excretory vessel in the posterior end of the scolex: *bm*, basement membrane; *dvm*, dorso-ventral muscles, $\times 1100$.
- Fig. 15. Proglottis 16 of a strobila, showing very early stage in the development of the reproductive organs: *agd*, anlage of the genital ducts, $\times 37$.
- Fig. 16. 17th proglottis of same strobila, $\times 37$. Figs. 15 and 16 are drawn from oil-of-cedar transparencies.
- Fig. 17. Transparent preparation of a mature proglottis,—testes and vitelline follicles not complete: *vs*, vesicula seminalis; *vd*, vas deferens; *cp*, cirrus-pouch; *co*, cirrus-opening; *vo*, aperture of vagina; *uo*, opening of uterus; *ov*, ovary; $\times 60$.
- Fig. 18. Transverse section through the interproglottidal region of the unjointed portion of the strobila, $\times 130$.
- Fig. 19. Same through the middle of the proglottis, only two eggs shown in the gravid uterus-sac, $\times 130$.
- Fig. 20. A single testis with its vas efferens: *cyl*, cytophore, $\times 365$.
- Fig. 21. Sperm-reservoir at the posterior end of the vas deferens, $\times 365$.
- Fig. 22. Anastomosis of vasa efferentia near the sperm-reservoir: *m*, median line of the proglottis, $\times 200$.

PLATE VI.

- Fig. 23. Cross-sections of vas deferens: *ev*, syncytial epithelium; *mcm*, myoblasts of circular muscles, $\times 1000$.
- Fig. 24. Cross-section of cirrus, $\times 500$.
- Fig. 25. Cross-section of younger cirrus, $\times 500$.
- Fig. 26. Longitudinal section of receptaculum seminis and first portion of spermaduct: *s*, sperms; *sd*, spermaduct, $\times 235$.
- Fig. 27. Genital ducts in the generative space, posterior view of a reconstruction: *cvd*, common vitelline duct; *od*, oviduct; *sd*, spermaduct; *oc*, oocapt; *yr*, yolk-reservoir, $\times 60$.
- Fig. 28. Ova, showing accessory cells in connection with two: *ac*, accessory cells; *cy*, cytoplasm, $\times 1000$.
- Fig. 29. Individual yolk-cells: *a*, from follicles; *b*, from a collecting yolk-duct, $\times 1000$.

- Fig. 30. Longitudinal section of the shell glands; *eod*, epithelium of the oviduct, $\times 1000$.
- Fig. 31. The uterus-opening: a, from a transparency; b, from a coronal section before the formation of the aperture, $\times 100$.
- Fig. 32. The egg, showing contained embryo, $\times 500$.
- Fig. 33. Another, showing the escape of the oncosphere surrounded by the ciliated mantle, $\times 500$.

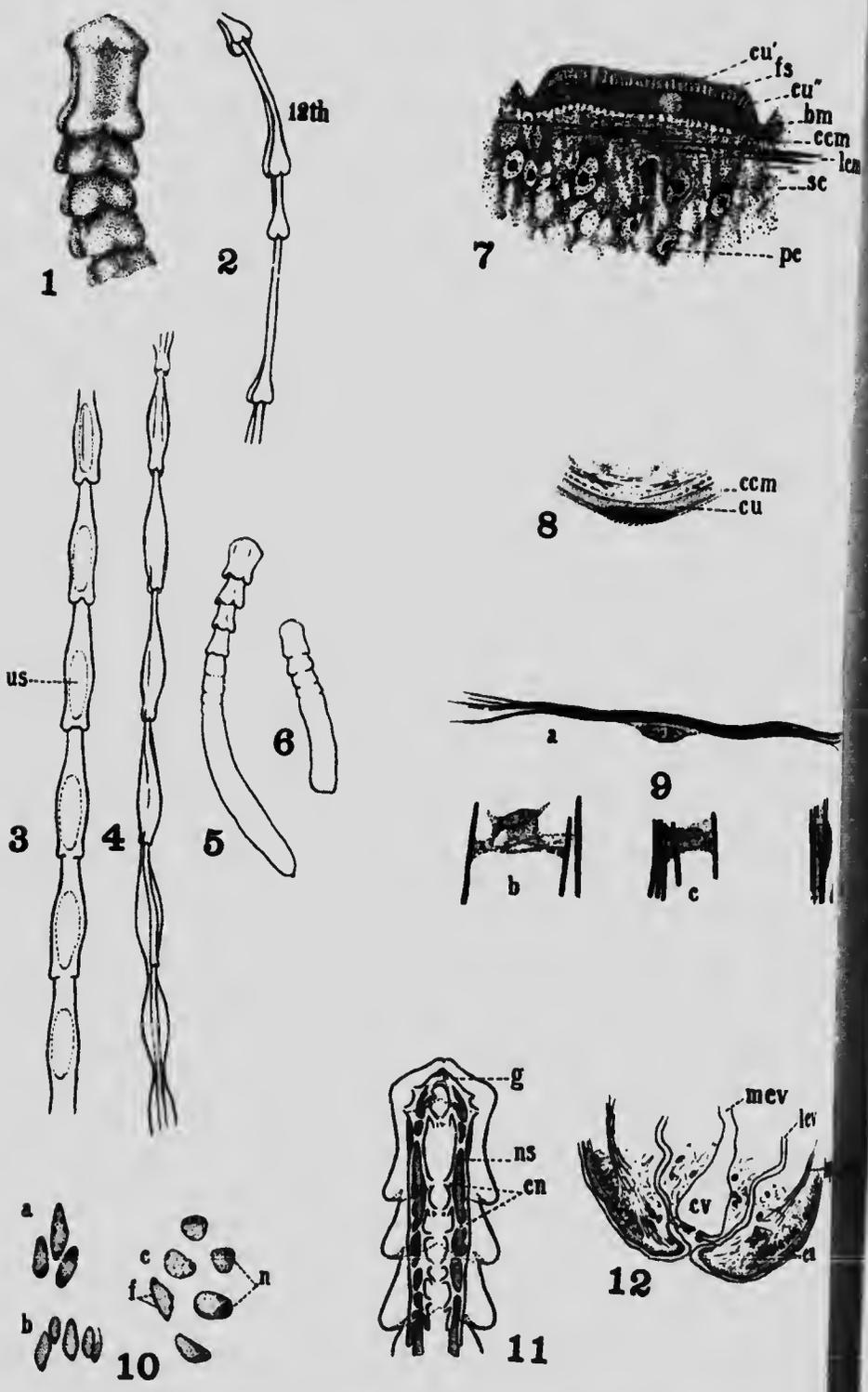
PLATE VII.

- Fig. 34. Photograph of a transverse section through the middle of the scolex: *ots*, outer transverse muscles; *b*, bothrium; *dvb*, dorso-ventral muscles of the bothria.
- Fig. 35. Transverse section through the posterior end of one of the foremost proglottides: *app*, auricular appendage; *ldvm*, lateral dorsoventral muscles.
- Fig. 36. Coronal section, slightly aside from the median line, through the scolex and first two proglottides: *ofs*, oblique fibres of the scolex; *cs*, cuticular spinules.
- Fig. 37. Similar section, in the median coronal plane: *aeo*, anterior excretory vesicle; *laf*, longitudinally arcuate fibers of the scolex; *mb*, bothrial muscles.
- Fig. 38. Portion of a transverse section through the cirrus-pouch: *de*, ductus ejaculatorius; *wcp*, wall of the cirrus-pouch.
- Fig. 39. Coronal sections through the vagina and its entrance, showing a migrating nucleus at *x*.
- Fig. 40. Portion of a longitudinal section through the vagina, showing two nuclei at *x* and *y* leaving the epithelium.
- Fig. 41. Another portion of the vagina, showing two nuclei passing through the basement membrane. Lettering as in the last figure.

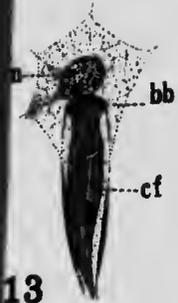
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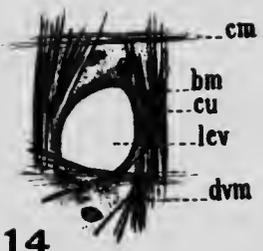




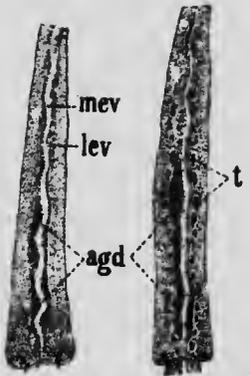
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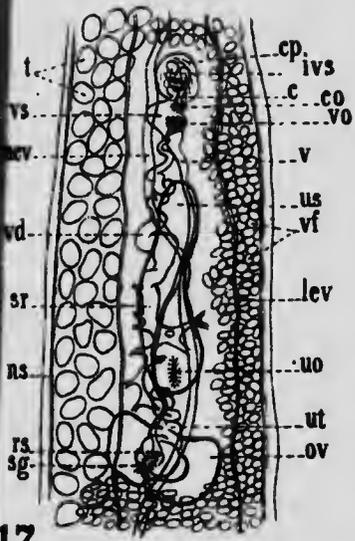


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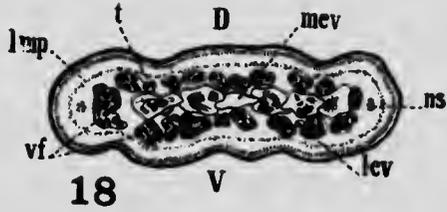


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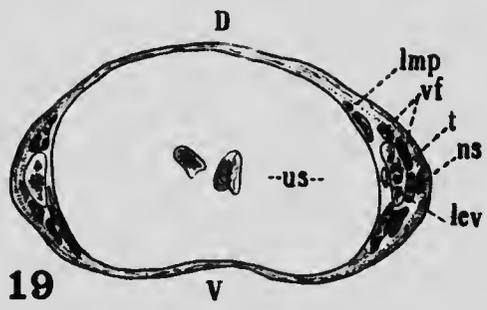
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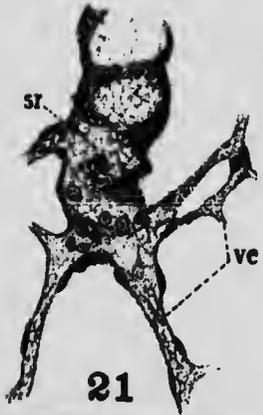
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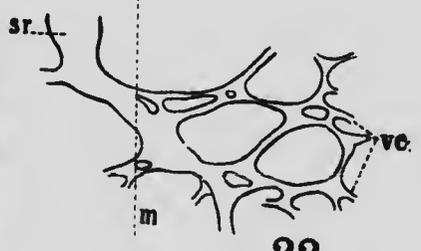
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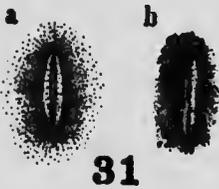
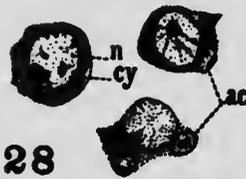
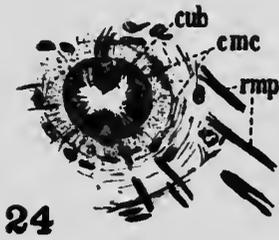
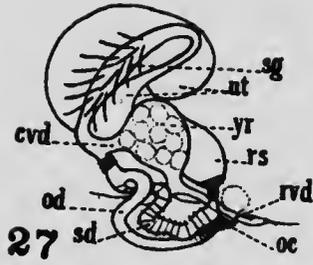
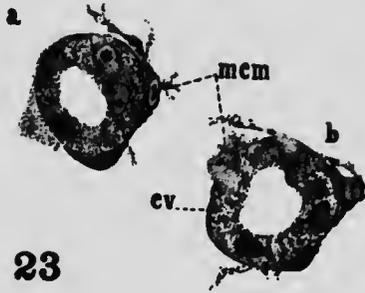


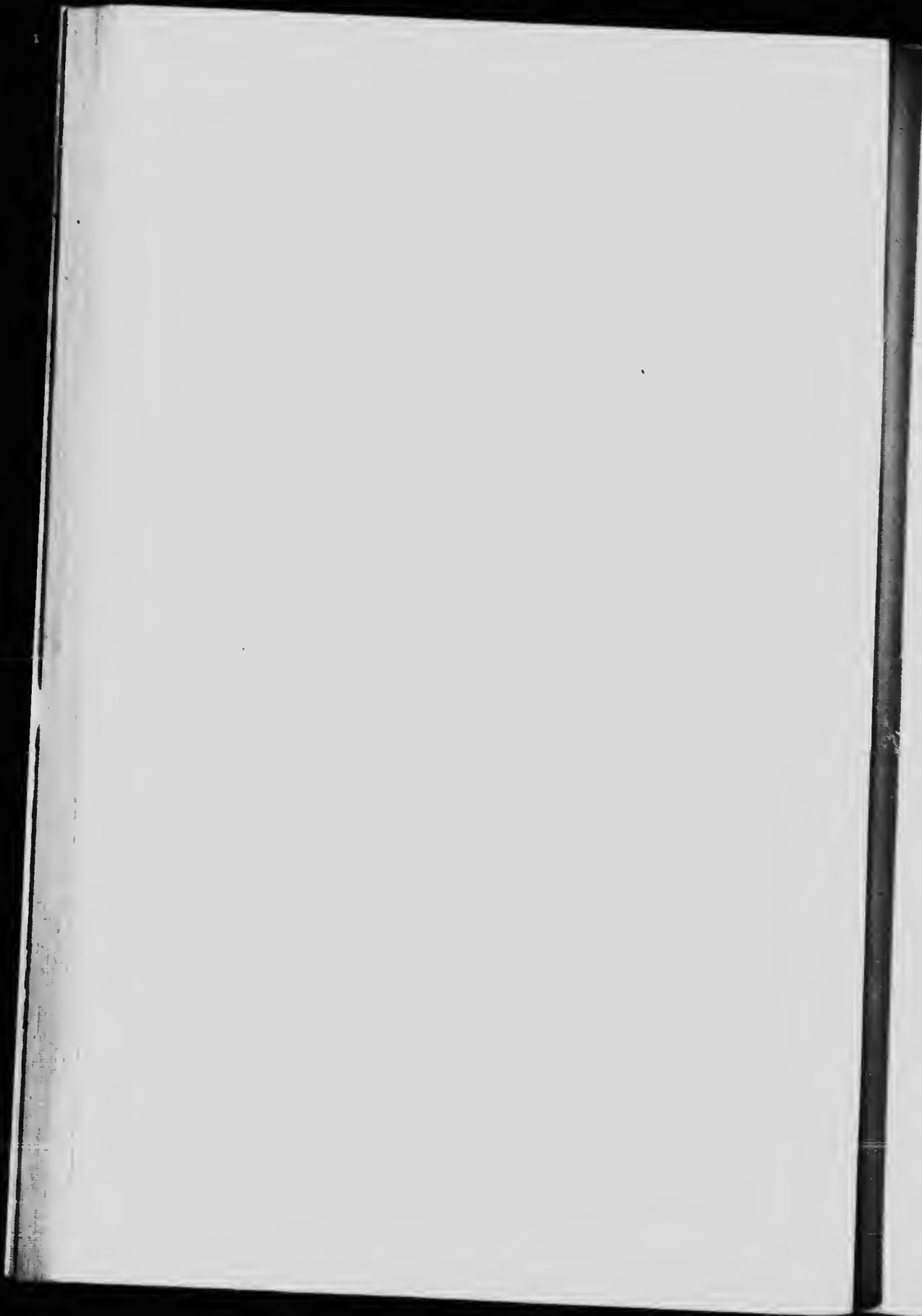
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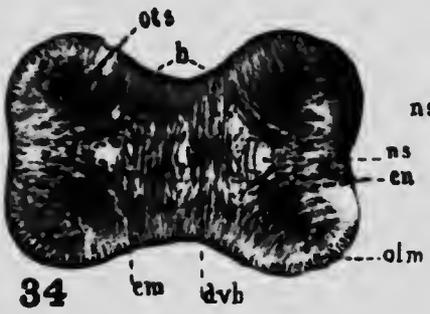


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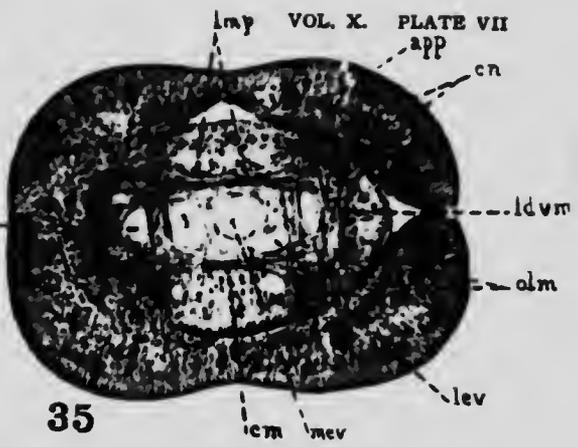




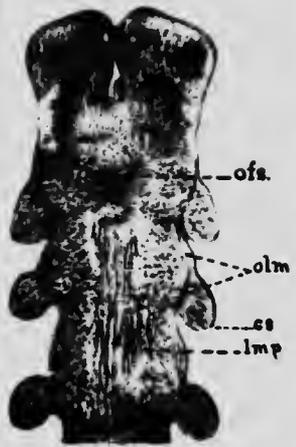




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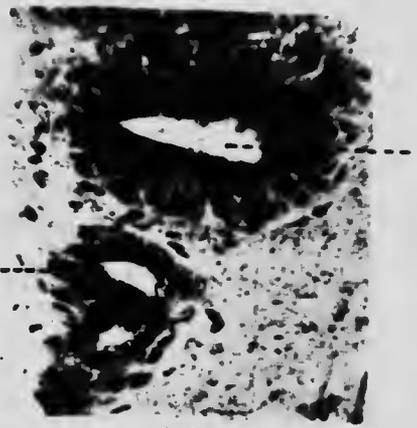
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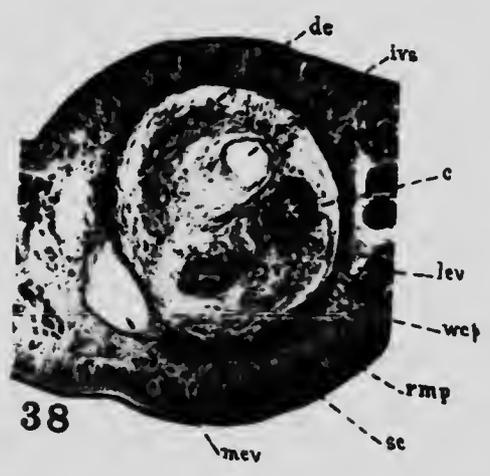
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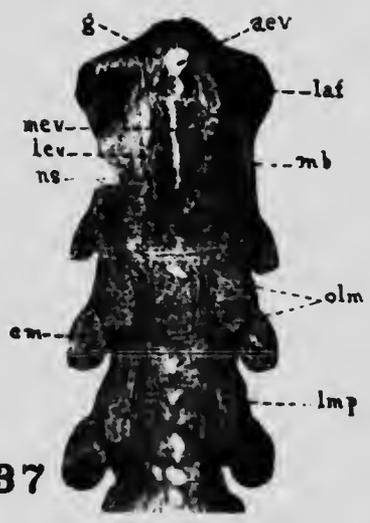
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THE EGG-LAYING HABITS OF *PLETHODON CINEREUS*

BY W. H. PIERSOL, B.A., M.B.

(Read 15th November, 1913.)

TWO accounts of the natural history of this, our commonest salamander, have appeared, one by Miss M. E. Cochran (1911) and one by the writer (1909). Both agree in their descriptions of the eggs, but neither gives any information as to the mode of deposition. The writer has sought to determine this by observations made on *Plethodon* both in its natural habitat and in a terrarium. The following is an account of the more important observations together with comment and inference. Fertilization is internal; this had been predicted in the earlier paper (1909) and has since been confirmed by the fact that a female isolated in a terrarium for four days laid eggs that developed naturally.

Case I. On one occasion the actual extrusion of the eggs was observed. The female had been placed when captured in a small glass jar along with fragments of the log in which she was found; and the jar with others containing eggs was carried back to the laboratory in a small bag. Chiefly for the sake of the eggs which are very delicate the bag was guarded from shocks as far as possible, then for another hour it stood unopened. On removing the jar from the bag it was seen that the egg laying had just begun, fortunately in such a position that all its details could be observed. The lips of the cloaca are pressed against the surface from which the eggs will eventually hang and a small quantity of mucus is extruded and adheres firmly to it. This much had been completed before observation began so nothing can be said as to the interval that then elapses before the first egg is laid. The extrusion of each egg occupies about twenty seconds and an interval of five to ten minutes occurs before the next appears. The first three eggs were laid in contact with the mucus above mentioned; the fourth, and last, adhered to them in turn through the stickiness of the egg-envelopes. As the female did not move during the entire process, all the eggs were laid at the same point, each egg as it came, crowding the preceding ones aside, thus making sure of being in contact with them. For over an hour after the last egg was laid the female did not change her position; during the next hour she left the eggs a few minutes, then returned and coiled herself about them.

The extrusion of the egg causes it to become elongated; the greater axis may be almost twice the less. In the case above noted the spherical form was assumed within a few minutes; in other cases the elongation has taken more than an hour to disappear. Exceptionally the elongated form may be retained for a considerable time. The most extreme case met with was an egg found among natural surroundings with the longest axis 5.25 m.m. and the shortest 2.75 m.m. In the same cluster was another elongated egg, its axes being 4.0 m.m. and 3.0 m.m. The three remaining eggs were spherical; all five were in the process of gastrulation. Another egg, quite similar to the one first mentioned was laid by a female in a terrarium; it kept pace in development with the remaining eggs of its cluster up to the 50-60 cell stage. In the first two cases the segmentation cavity had formed near one end of the long axis, in the third case near one end of a short axis. As the eggs were fixed at the stages mentioned it is impossible to say how the further development would have been affected.

This mode of egg-laying places *Plethodon* at the end of a progressive series, the most primitive member being *Cryptobranchus*, with eggs laid in a uniform rosary-like string as described by Reese (1904) and Smith (1906). Next, as suggested by Wilder (1913), would stand *Desmognathus*; in this genus most of the eggs have left the main string of the rosary and lie at the sides of it, each retaining connection with it, however, by a short stalk. The next step is represented by such a case as *Spelerpes* (Wilder, 1899) or *Antodax* (Ritter and Miller, 1899); here the disappearance of the main string leaves each egg to be attached separately to its support—usually a stone—by a short stalk. The disappearance of this stalk for each egg, except the first, produces the separate eggs of *Plethodon*. This economy of material is highly desirable in so small an animal. The position of *Antodax* in the series given above is not that usually occupied by the genus in a series that shows progressive modification of some primitive habit; in most respects *Antodax* has departed furthest from the primitive amphibian mode of life, and *Plethodon* can only offer suggestions as to the path along which *Antodax* has travelled to its present condition. In habits, however, as in morphology, it does not follow that the higher member of a series must in every point have progressed beyond the lower.

Other observations differing from the foregoing are as follows:

Case II. In examining a terrarium on one occasion there was uncovered a female that had evidently just completed the extrusion of the eggs. Two eggs, approximately spherical, were in contact and cohering slightly; four other eggs, each more or less elongated, were lying separated from each other by intervals of about one-quarter of an inch; none

of them were suspended. Evidently under the somewhat unnatural conditions the female had moved after the extrusion of each of the last five eggs. *Case III.* In picking apart a decaying log there were exposed on one occasion a female and four eggs. One of these lay by itself, markedly elongated; the other three were in contact, two of them somewhat elongated, the third apparently spherical. All four were lying on the floor of the cavity, which fortunately had been opened from the side. Examination of the female revealed the existence of four eggs in the posterior parts of the oviducts. Evidently the egg-laying process had been interrupted by the opening up of the nesting-chamber.

These last two cases have been selected from among a few of the same general character because they differ from the rest in that the eggs were not suspended. In opening up logs a few clusters have been found unattached. At first, in such cases, it was taken for granted that the opening up of the nesting-chamber had involved the loosening of the eggs. Since attention has been directed to the possibility of a cluster not having been attached, two such have been found under circumstances that would seem to preclude the idea of their having been torn from their attachment. In neither of these two cases could a stalk attached to the cluster be found. It would seem that occasionally the tendency to reduce the amount of material devoted to forming stalks for the eggs goes so far as to eliminate even the stalk of the first egg. No exact count has been kept of the number of such cases as compared with the normal, attached ones, but the impression left is that it is very small.

As might be concluded from *Case I*, an examination of the relation of the stalk to the eggs shows that it does not come from any one particular egg, but from a quantity of mucus that adheres to the outer envelope of certain of them; the impression given is that of a material poured onto the bunch, part of it being drawn out to form the stalk. As is the usual case among Urodeles the outer envelope of each egg is of a much more sticky mucus than the inner ones. *Plethodon* is peculiar in having this outer layer unusually thin, and in depositing a still more sticky mass of mucus before the egg-laying proper begins.

In most amphibia the impulse toward the deposition of the eggs, once these are ready for the act, is an imperative one. In some cases (e.g., many frogs) the assistance of the male is needed, but generally speaking, when the proper time comes the spawn will be deposited even with conditions and surroundings that are far from natural. Both *Rana pipiens* and *Rana catesbiana* that have been kept over winter, without feeding, in a tank in the basement of the Biological Building of the University, have been known to spawn in spring and early summer respectively. (Such spawn has never developed, evidently has never

been fertilized.) In *Plethodon* the instinct is more delicately adjusted. This is shown in the marked preference for some particular log as a site for egg-laying. For instance, one small plot of woodland was found to contain *Plethodon* in abundance during the spring of 1913 and was visited on June 21st in the search for eggs. A dozen or more rotting logs yielded only males or sexually immature specimens; at last one log was found which, though apparently not differing from the others, yielded eleven females with eggs. A number of similar cases have been met with. The logs so greatly preferred are invariably conifers, but other factors must enter into the quest on for another coniferous log that seems quite similar may be close at hand yet be entirely destitute. Equally striking is the difficulty that has been experienced in getting females to lay eggs in a terrarium. The thin, almost translucent ventral wall of the abdomen allows the easy recognition of females containing eggs almost ready for deposition. If pieces of the logs in which the animals have been found are brought from the field and the pieces piled together in a terrarium so as to reconstruct roughly the log, there is no difficulty in keeping the animals alive and in good condition for long periods. They will feed readily on small insects, e.g., aphids; but, like most amphibia, seem to suffer little from long deprivation. Three specimens overlooked in a small terrarium last spring lived until the end of September with no attention; at the end of that period their physical condition and vigor had suffered so little that they could not be recognised after being allowed to mingle with others brought in from the field. In spite of this apparent easy acceptance of life in a terrarium, the change usually is sufficient to inhibit the egg-laying reactions, and the eggs are retained and absorbed during the next five or six weeks. Exceptionally they will be laid as under natural conditions, but only when the female has been brought from the field not more than three or four days before the time for egg-laying. It is not a question of previous impregnation or its lack, for as far as examined, all mature females have been found to have the receptacles filled with sperm some time before the egg-laying season arrives.

The character of the season has some influence on the depth beneath the surface at which the eggs are laid; in damp seasons they will be for the most part but an inch below the surface, in dry seasons they will be four or five inches below. This refers to the character of the season up to the time of egg-laying, not after.

The retention of one egg in the ovary was mentioned in the earlier paper. Later experience has confirmed the observation. The egg is always much under-sized and occurs in about one third of the females accompanying clusters of eggs in early stages of development; it is then rapidly absorbed, and must have considerable value as a

supply of nourishment for the female during her wait by the eggs. Occasionally it will almost equal the remaining eggs in size and then will be laid along with them, producing a cluster with one markedly small egg. For example, in one cluster of seven eggs, six of them had a diameter of 3.75 m.m., the remaining one of 2.75 m.m. From a difference so marked as this there is a gradual transition to the state where all the eggs of the cluster are the same size; such are about one half of all cases. The writer has twice found similarly undersized eggs of *Amblystoma*; the numbers were small, nine and eleven in the two cases, and the eggs of but two-thirds the normal size. They developed normally, producing under-sized larvae which were perfect anatomically but defective in their feeding instincts. The one lot would not feed at all; the other would snap fitfully at *Cyclops*, etc., but would not eat enough to grow or ever to maintain life. This was quite striking for both lots were the species *jeffersonianum* the larvae of which are normally voracious feeders and easy to raise. In *Plethodon* the early development of the small egg is quite normal, its fate has never been followed past the time when the larva is well formed.

One female, kept in a terrarium with her eggs, swallowed two of them, and three hours later regurgitated them. The eggs were killed by the process, whether by digestive action or by the mechanical violence it is impossible to say, for they were in the process of gastrulation at the time. This is a most critical period for the egg, its delicacy is at the maximum and very slight disturbance will cause its death. The swallowing of their spawn has been noted for many amphibia, usually where, as above, something has happened to pervert the natural instincts. Smith (1907) however, describes it as normal for *Cryptobranchus*; in this case moreover when regurgitated the eggs frequently continue to develop.

Means taken to determine the mating habits have so far been fruitless. The single observation of Wilder (1913) on *Desmognathus* is probably a close approximation to the habits of *Plethodon* in this respect.

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