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# On Two Species of Aquatic Oligochæta,

## *Limnodrilus gotoi* HATAI and *Limnodrilus willeyi*, n. sp.

By

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*With 34 figures in text.*

### Introduction.

Since the appearance of Dr. HATAI's paper (1899) upon *Limnodrilus gotoi*, which was abridged and cited the next year by Dr. W. MICHAELSEN in 'Das Tierreich—Oligochaeta (1900),' it has been generally recognised as a distinct species. Some time ago specimens of an Indian *Limnodrilus*, which showed some characteristic resemblances to *L. gotoi* HATAI, were handed over by Dr. WILLEY, then the Director of the Colombo Museum, to Prof. WATASÉ, with the suggestion that Prof. GOTO or Dr. HATAI study and report on them. These specimens were committed to me by Prof. GOTO, who also suggested the investigation of several questions on aquatic oligochætes. My studies on the subject have brought out two principal results of systematic importance, which may be stated at once as follows:

1. *Limnodrilus gotoi* HATAI includes two species—*L. gotoi* and *L. willeyi*, n. sp.
2. *Limnodrilus gotoi* is also found in India.

Before I enter into the subject of this paper I must express my best thanks to Prof. Goro for his kind supervision and helpful advices. I must also note that the work was done in the Zoological Institute of the Science College, Imperial University of Tokyo.

I have found three species of *Limnodrilus* among my collections of aquatic oligochætes, which were made in the gutters and ditches of Tokyo and Morioka from July to November, 1910. They differ distinctly from one another by the length of the chitinous penis sheath. To be more precise, in Form A the chitinous penis sheath is 3–4 times, in Form B 10–11 times, and in Form C 30–33 times, as long as the diameter of the proximal end. There were no intermediate lengths, and the three forms differ moreover in several other points.

The Form C does not come into consideration in the present paper. The forms A and B must now be compared with *L. gotoi* HATAI as to their systematic characters, which may be shown, for convenience, as follows.

| | Form A | Form B | L. gotoi HATAI |
|---|---|---|---|
| Spermathecæ | with spermatophores | without spermatophores | with spermatophores |
| Prostata | spindle-shaped | irregular shape | spindle-shaped |
| Penis sheath | 3–4 times as long as wide | 10–11 ditto | ? 10–11 ditto |
| Ant. sperm-sac | unpaired | paired | unpaired |
| Post. sperm-sac | XI only, unpaired | XI–, unpaired | XI–, unpaired |
| Setæ | sigmoid with unequal branches | sigmoid with about equal branches | sigmoid, bifurcate |

The generic characters are not included in the above table. The

measurement of the chitinous penis sheath of *L. gotoi* HATAI is not given in the original paper, but the author gives among others two different figures of it—one like that of Form A (A. Z. J. III., tab. II., fig. 8) and the other closely resembling that of Form B (l. c. fig. 10). From these figures we may infer that the chitinous penis sheath of *L. gotoi* HATAI is either 4 or 10–11 times as long as it is broad at the proximal end.

Now an examination of the above table shows that, *L. gotoi* HATAI resembles Form A in the prostata, anterior sperm-sac and spermathecæ, and Form B in the chitinous penis sheath and posterior sperm-sac.

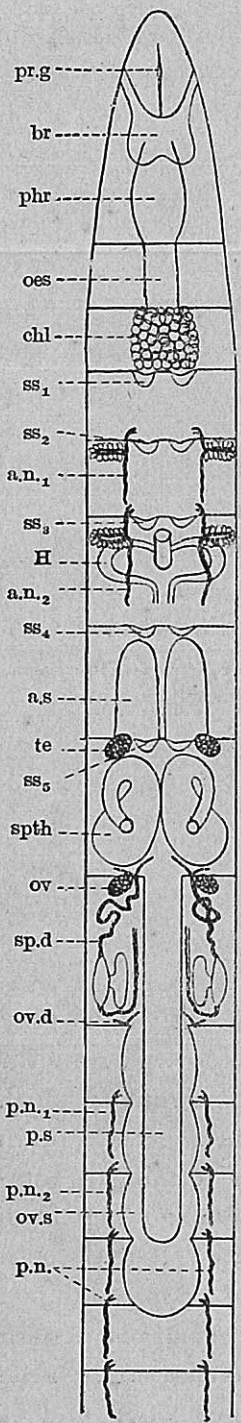
Here it must be repeated that the three species of *Limnodrilus* above mentioned are the only ones represented in my fairly extensive collections made in different localities in Tokyo.

These facts appear to me to prove that HATAI has confused two species into one. I will, therefore, propose the name *Limnodrilus willey*, n. sp., for Form A, retaining that of *Limnodrilus gotoi* for Form B. The new name is in honour of Dr. A. WILLEY, to whom I owe my Indian specimens.

## PART I. *Limnodrilus gotoi* HATAI 1899 NOMURA emend.

### 1. Description.

Colour of the body red, shaded posteriorly with light gray or yellow. Very fine transverse striations, due to septa, in the posterior part of the body. Prostomium somewhat conical. Segments I–V always biannulate, anterior annulus shorter. Brain almost square, emarginate posteriorly. Clitellum forming a complete ring, usually in segment XI, but sometimes extending from segment ½X to segment ½XII, or from segment XI to XII, both entirely inclusive. Setæ sigmoid, bifurcate at end; lower branch strongly and upper slightly curved; both almost equal in length under a low power. Each seta-bundle mostly consists of 6 setæ in the anterior segments, of 3–5 in the middle segments, and of




1–2 in the posterior. Five pairs of septal sacs ($ss_{1-5}$) attached to the posterior faces of septa V/VI–IX/X, on the ventral side of the intestine. Pharyngeal glands very weakly developed. Pharynx in segment ½ II and III. Œsophagus in segment IV. Intestine and chloragogues commence in segment V. The nephridia ($a.n._{1.2}$) of segments VII and VIII are racemose in appearance. Prostata rather roundish, entirely surrounding the atrium. Chitinous penis sheath 10–11 times as long as its diameter at the proximal end; circular in cross section all through. Anterior sperm-sacs (a.s.) in segment IX, paired. Posterior sperm-sac (p.s.) in segments XI–? XVII, unpaired. Spermathecæ (spth) in segment X, paired; no spermatophores have been observed. Body length 70–100 mm. according to seasons. Number of segments 100–150. Hab. — Morioka; Tokyo; Kagoshima; Colombo, Ceylon.

## 2. Anatomy and Histology.

For the purposes of this section 6 Japanese and 20 Indian specimens were

Fig. 1.

*L. gotoi.* Diagram showing the principal organs. Behind segment V the intestine is omitted. $a.n._{1.2}$ — anterior nephridia, a.s—anterior sperm-sac, br—brain, chl—chloragogues, H—heart, oes—œsophagus, te—testis, ov—ovary, ov.d—oviduct, ov.s—ovisac, phr—pharynx, $p.n._{1.2}$—posterior nephridia, pr.g—prostomial ganglion, p.s—posterior sperm-sac, sp.d—sperm-duct, spth—spermatheca, $ss_{1-5}$—septal sacs.

used. The Indian material which was fixed with either sublimate or formalin gave tolerably good results. The Japanese materials were killed, after narcotising with dilute alcohol, with acetic sublimate and PERENYI's fluid. For histological studies sections were cut 4–7 $\mu$ thick.

The stains used were 1) DELAFIELD's hæmatoxylin and eosin, 2) MALLORY's connective tissue stain, at the suggestion of Prof. IJIMA. For general observations 1) was more suitable, but 2) gave excellent results for minute details, though less favourable for study under a low power.

## 1. BODY WALL.

The body wall consists of 5 layers.

1). The cuticle is very thin and structureless. I have not been able to find any striations or other markings, nor the large pore canal figured by VEJDOVSKÝ in *Limnodrilus udekemianus* CLAP. at the most anterior part of the prostomium. According to L. ATHESTON, the cuticle of *Tubifex rivulorum*, when isolated, shows openings or markings of two sorts. "These are probably the pore canals from the gland cells and the openings occupied in the living condition by the hairs from the sense cells." He used dilute KUSKOW's fluid for the purpose of maceration.

In fresh materials left in a 3% aqueous solution of caustic potash for about two days, I found, though with great difficulty, many pore canals in the cuticle of the clitellar part and other regions, but the openings of the other sort could not be observed.

At the two ends of the body the cuticle is reflected inwards and is continued into the mouth and anal cavity.

The cuticle is thickest at the clitellar part and becomes thinner at both ends of the body. At the clitellar part it is 1.3–1.5 $\mu$ thick, in the neighbouring segments 1.0–1.2 $\mu$; at the most posterior part and in the mouth cavity 0.5–0.8 $\mu$.

2). The hypodermis consists of two well marked types of cells, viz. gland cells and columnar hypodermal cells. The sense cells which are undoubtedly present could not be well observed

in my sections. The clitellar hypodermis is notably different from that of the other parts.

*a.* Ordinary, or extra-clitellar hypodermis. This layer is generally 5–6 $\mu$ thick, but near the two ends of the body it is thicker. At the most anterior part the cells are columnar or sometimes fusiform, 10–15 $\mu$ long and 4–5 $\mu$ across. At the most posterior part they are nearly cubical, measuring 12–13 $\mu$, and the cytoplasma shows but little differentiation.

"At a distance of about 4 millimeters from the (hind) end", writes ATHESTON, "the body wall merges into a mass of cells lying against the great dorsal blood vessel and constituting the growing zone; anterior to this, the body wall is marked off into its distinct layers." A similar condition occurs also in *L. gotoi.*

Outside the two thickenings at the ends of the body the hypodermal cells are flattened and the cell-boundaries are indistinct, especially in sections stained with eosin. They are apparently supporting cells and the syncytial condition is undoubtedly a secondary result.

The cytoplasma of the hypodermal cells is always exceedingly compact. In the anterior and middle parts of the body, the nuclei are more or less elongated ellipsoidal or fusiform, and rarely contain a single nucleolus. Near the posterior end the nuclei are roundish and always contains a large nucleolus.

The gland cells are large and contain a small amount of feebly staining cytoplasma. They are very abundant in the anterior half of the body, but very few in the posterior, and they do not show any regular arrangement, two cells sometimes uniting into one.

*b.* Clitellar hypodermis. In life the clitellum is distinguished from the other parts by its glandular appearance. The gland cells are 20–23 $\mu$ long and 8–10 $\mu$ across, and three well marked stages can generally be observed in fully mature specimens, viz. a highly vacuolated condition, a more or less granulated condition and a condition in which the cells contain many large globules, 2 $\mu$ in diameter. Besides these gland cells there are, between them, very slender, rather fibrous cells. The nuclei of the clitellar

gland cells are large and contain a single nucleolus, usually lying near the base.

3). On the circular muscle layer I have but little to add to the description of HATAI. I must however note the presence of compact granular bodies between the circular muscle fibres, which take up orange G in MALLORY'S method. In eosin stained sections they appear as diffuse granules. Their nature remains totally unknown to me.

4). Immediately under the circular muscle layer run the longitudinal muscle fibres parallel to the body axis. It is divided by lateral lines into two bundles, the dorsal and the ventral (HATAI). Sometimes the boundary between the two disappears. This layer is very weakly developed at the posterior end of the body.

5). The peritoneal layer consists of large vesicular cells, and stands in close relation to the longitudinal muscle layer. It is strongly developed in segments IV–VIII, but less well in the prostomium and the posterior segments of the body. The cells of the peritoneum are irregular in shape, and the layer always presents a smooth surface toward the body cavity. The nuclei are large and sometimes nucleolated. The cells of the lateral lines are richer in cytoplasm than the neighbouring peritoneal cells.

## 2. SETÆ AND SETA-BUNDLES.

The sigmoid setæ of *L. gotoi* are nodulate and bifurcate at the distal end. The branches appear almost equal under a low power, but unequal under a higher. In the anterior parts of the body the upper branch is larger than the lower, but in the posterior parts the reverse is the case. The nodulation is smooth. A seta passes through the cuticular layer at a point which lies between the nodule and the tip. In the anterior parts of the body the setæ "are arranged in bundles in longitudinal lines corresponding to the four corners of a squarish cross-section of the body" (HATAI), but in the posterior parts the dorsal and the ventral seta-bundles of each side approach the lateral line

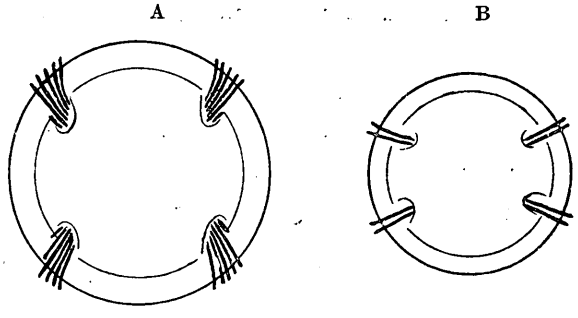



Fig. 2.

*L. gotoi.* Diagrams of cross sections of body, showing the position of the seta-bundles. A—anterior segment, B—posteriormost one.

(fig. 2). "They are present from the second segment and their position in each segment is rather posterior than middle" (HATAI). As already mentioned, the anterior five segments are always biannulate, and in these segments the seta-bundles are borne at the middle of the posterior annulus. Each seta-bundle is always accompanied by one or two young setæ and the posterior bundles often consist of young setæ only. In segment XI the ventral seta-bundles are absent.

The setigerous follicle is formed of cells derived from the hypodermis by proliferation, and the initial stages of its development can be seen at the most posterior part of the body. "The setæ first appear as small cones of chitinous substance" (BEDDARD) at the bottom of the organ, and as far forward as 2–3 mm. from the hind end of the body, the setæ, growing in length and pushing out through the tissues, begin to break through the cuticular layer, which is at first lifted up by the growing seta.

The follicle cells of the full grown anterior setigerous organs undergo a sort of chitinous metamorphosis and present very fine striations parallel to the setæ. In the follicular hypodermis there are neither cell-boundaries nor nuclei, the latter having wandered into the follicle and assumed a more or less regular arrangement. In these full grown setigerous organs, both the seta-forming and young follicle cells are observable as parts of the follicular fundus. This part lies at first close to the median plane of the body, but

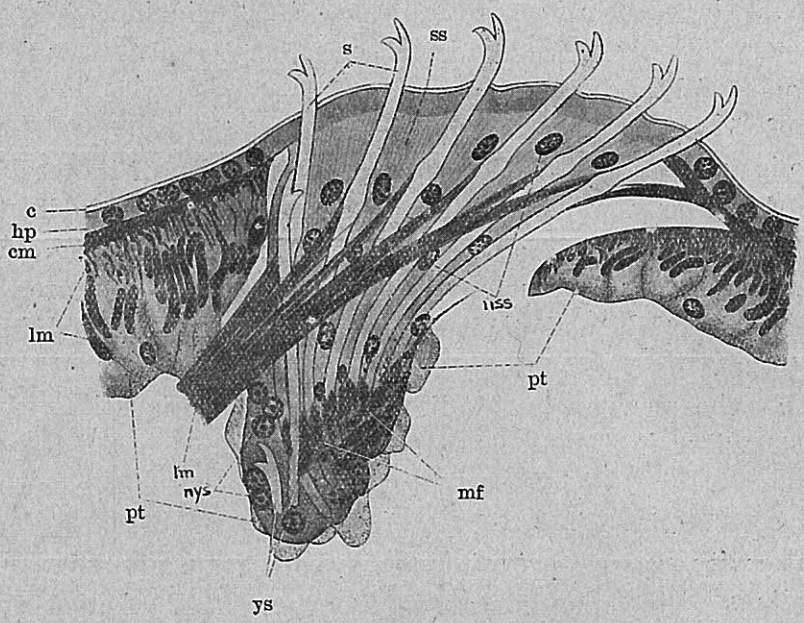



Fig. 3.

*L. gotoi.* Cross section through a fully grown setigerous organ of left dorsal side. ×470. c—cuticular layer, hp—hypodermis, cm—circular muscle layer, lm—longitudinal muscle layer, pt—peritoneum, mf—parieto-vaginal muscle fibres, tm—interfollicular muscle, ys—young setæ, s—setæ, ss—setigerous follicle, nys—nuclei of young follicle cells, nss—nuclei of setigerous follicle.

gradually move outwards with the completion of succeeding setæ, so that the organ becomes flattened antero-posteriorly and is spread out sidewise. The arrangement of the setæ in an organ is like that of the sticks of an open fan. In the seta-forming portion of the organ the cell-boundaries are also invisible, and large round nucleolated nuclei are found scattered in it.

In fully formed setigerous organs there are two sets of muscles—the parieto-vaginal and the interfollicular, by which the seta-bundles are moved similarly as in other oligochætes.

## 3. Alimentary canal.

The alimentary canal of *L. gotoi* consists of four parts as in other tubificid oligochætes. They are the mouth and buccal cavity, pharynx, œsophagus, and intestine.

1). The mouth lies ventrally at the beginning of the first segment. The buccal cavity is flattened dorso-ventrally and ends in segment ½ II, becoming smaller and ⊥-shaped. The wall consists of 5 layers corresponding to those of the body wall. The cuticle is as already mentioned a direct continuation of the cuticular layer of the body wall, but there are no pore-canals in it. The hypodermis does not contain gland cells but is otherwise similar in structure. The circular and longitudinal muscles are very weakly developed, and the peritoneum is also changed into a solid tissue.

2). The pharynx is the direct posterior continuation of the buccal cavity; it begins in segment ½ II and reaches to the end of segment III. The inner cavity is very spacious, except at the anterior part, where it is very narrow, and the ⊥-shape of the mouth cavity passes over into an irregularly penta-radiate form by foldings of the pharyngeal wall. About the middle of the pharynx, where the cavity is broadest, the lumen again becomes ⊥-shaped. At the posterior part the median inlet disappears more or less suddenly and passes over into the dorso-ventrally flattened, but laterally extended œsophagus. In completely narcotised specimens the median inlet is not very deep and the entire cavity has somewhat the form of a semicircle in cross-section.

The wall of the pharynx appears to consist of two layers. The inner or endodermal layer consists of exceedingly long, cylindrical, ciliated cells; the cytoplasm is somewhat granulated and an exceedingly elongated nucleus lies at the middle or in the proximal half. The cilia are stouter on the dorsal than on the ventral side. The outer layer is made up of several elements, viz. exceedingly fine blood capillaries, pretty well developed circular and very weakly developed longitudinal muscle fibres, and connective tissue between them. Besides these there are muscle fibres running between the pharyngeal and the body wall.

On the dorsal side of the pharynx and attached to its wall there are certain cells with dense cytoplasm, which are not chloragogues. These cells, which are pharyngeal glands, are also present in *L. willeyi*, as will be seen later.

3). The œsophagus is confined to segment IV. Its lumen is flattened dorso-ventrally and its wall is similar in structure to that of the pharynx, except that the endodermal cells are not so long, and the muscle fibres extending between the alimentary canal and the body wall are absent. The chloragogue cells are rarely seen attached to the wall.

4). The intestine begins in segment V. In cross-section its lumen, for the greater part, is capacious and nearly circular, and is constricted by the septa at the intersegmental lines; but in the posterior part, which is shorter, it becomes tall pentagonal in outline and finally squarish. The anus is dorso-posterior in position.

The intestinal wall apparently consists of three layers, viz. endodermal epithelium, vascular layer, and chloragogue layer. The ciliated epithelial cells are large, measuring 20–25 $\mu$ by 7–10 $\mu$, and have a compact cytoplasm. The nuclei lie more in the distal half of the cells, and contain each a nucleolus. With MALLORY's stain two kinds of epithelial cells come to view. One shows a great affinity for anilin blue and the other for orange G, and the interesting points are that the cilia of the latter cells are stained more deeply blue than those of the former, and that the blue stained cells appear to be more glandular than the orange stained. There are also others of intermediate character. It is therefore very probable that these cells are different stages in the activities of the epithelial cells.

The epithelial layer of the intestine is closely followed by the vascular layer, and outside the latter lie the intestinal muscle layers. The intestinal muscles are also disposed in two layers like those of the body wall, viz. the inner circular and the outer longitudinal. They are both feebly developed and the individual fibres stand apart from one another.

The chloragogues are, as is generally supposed, but modifications of the peritoneal epithelium. The chloragogue layer is well developed in the anterior segments, especially in V–VIII. In the posterior segments, however, the cells lose their characteristic appearance and become ordinary connective tissue layer, as around the pharynx and the œsophagus.

The typical chloragogue cells are club-shaped, with the thicker end directed towards the body cavity, and the cytoplasm is thin. The nuclei always lie in the distal half of the cells and contain each a large central nucleolus. The cells invariably contain chloragogue granules and vacuoles. The chloragogue granules are perfectly round and of a blackish brown colour in life. In dark coloured specimens the cells are richly filled with these granules. The granules are large and stain well with the fuchsin of MALLORY'S method and HEIDENHAIN'S hæmatoxylin. They are of uniform size and measure 1.3 $\mu$ in diameter. The vacuoles are of different sizes, some being half as large as a cell. If a single large one is present in a cell, it always occupies the proximal half. In life the whole vacuole is filled by a fat or oil globule.

## 4. SEPTA, SEPTAL SACS AND AMŒBOCYTES.

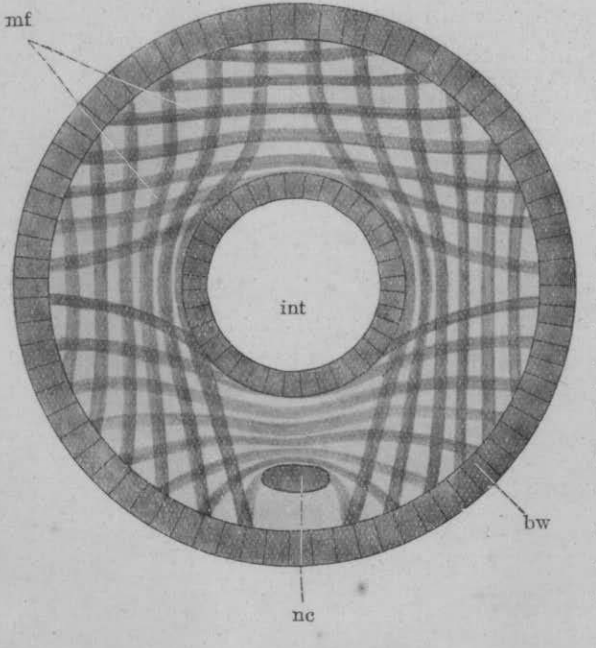



Fig. 4.

*L. gotoi.* Diagram showing the septal muscles. bw—body wall, int—intestine, nc—ventral nerve cord, mf—muscle fibres.

The cœlom is very spacious and traversed by the intersegmental septa, which begins at III/IV. In the anterior part of the body the septa are always more or less funnel-shaped, with the smaller end directed posteriorly, especially in the fore part of the genital segments. The septa X/XI and XI/XII are often exceedingly thin, and are apt to be overlooked in fresh specimens.

Each septum consists of three layers.

The middle layer is that of the muscle fibres, whose arrangements are shown diagrammatically in fig. 4. They branch and reticulate in various ways, and terminate in the circular muscle layer of the body wall, after separating into a number of fibril-bundles at the two ends. The peritoneal cells that cover up the muscle layer on either side are exceedingly flattened, but are otherwise similar in appearance to those of the body wall.

In the posterior septa of the body there occur, here and there, cells with compact cytoplasm, less flattened and rather spindle-shaped, with a thin cell wall and containing each a large nucleolated nucleus. These cells are different from the amœbocytes and the peritoneal cells, and are probably neoblasts, which take an active part in regeneration.

Near the posterior end of the body, the septa become more or less incomplete and still more posteriorly they are represented by undifferentiated cell-strands.

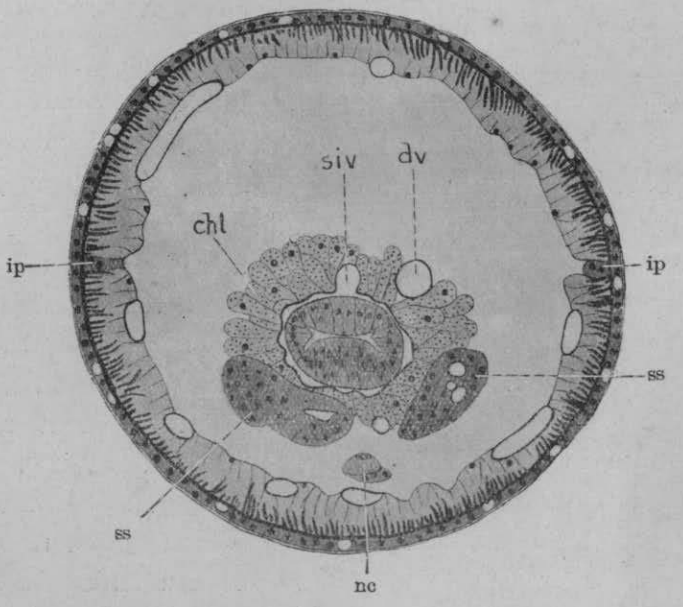



Fig. 5.

*L. gotoi.* Cross section through segment VI to show the position of the septal sacs. × 150. chl—chloragogues, dv—dorsal vessel, ip—lateral line, nc—ventral nerve cord, siv—supraintestinal vessel, ss—septal sac.

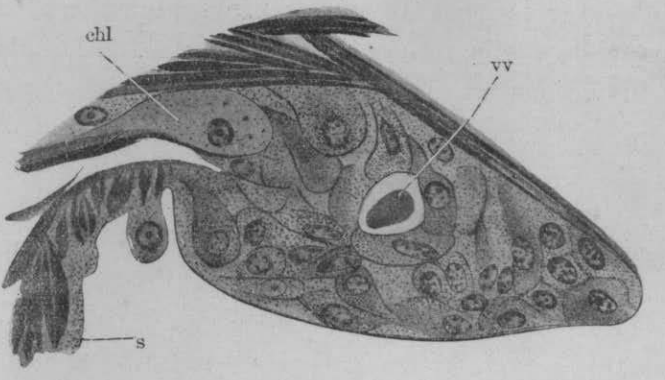



Fig. 6.

*L. gotoi.* Longitudinal section of a septal sac through about its middle. ×700. s—septum, vv—ventral vessel, chl—chloragogue cell.

The septal sacs, which are outgrowths of the septa, are attached in pairs to the posterior faces of septa V/VI–IX/X, on the ventral side of the intestine. The sac is provided with a thin structureless membrane, and always contains many club-shaped cells, between which certain interesting relations can be made out. The cells, which are seen at the entrance of the sac, are undoubtedly chloragogues, while those at the bottom are smaller and have a dense cytoplasm; and between these two forms there are cells of intermediate characters. Confining our attention for the present to the septal sacs, the conclusion is justified that one sort of these cells is derived from the other, and as to the relation of these cells to the chloragogues, there are two possibilities: either that the chloragogues change into the bottom cells, or the reverse is true. At present I have not sufficient data to decide between these two alternatives; but one fact appears to me clear, that the septal organs have an intimate relation to the ventral blood vessel passing through them. Each bottom cell always contains a single nucleus, which is somewhat elongated and rarely nucleolated; the presence of a cell wall is very doubtful.

Before leaving the septal sacs I must make one or two remarks on the septal glands described by HATAI. He says,

"Two pairs of septal glands perhaps continuations of the cœlomic epithelium, are attached to the posterior faces of septa VII/VIII and VIII/IX, on either side of the median line. The form and size of their cells are closely similar to those of the peritoneal cells. These cells are aggregated around one axis." This description recalls to one's mind, with some uncertainty, the septal sacs which are found attached to the posterior faces of septa V/VI–IX/X; but the following statements do not apply to the organs observed by me. "The axis is a continuation of the peritoneum and originates a little in front of the ventral setæ and is in the same longitudinal line with the latter." The septal sacs may possibly be described as lying in the same longitudinal line with the seta-bundles, but it never extends so far as to lie close to the setæ, and moreover no other similar organs are to be found in segment IX. I entertain the opinion that the organs described by HATAI may possibly be the anterior nephridia. In that case the numbers of the septa must be misprints.

In the body cavity lie amœbocytes, attached to the intestinal wall or to the peritoneum of the body wall. Each amœbocyte is a multinucleated, naked mass of cytoplasm of irregular shape, sometimes sending out pseudopodia-like processes. They are also often found floating free in the cœlom, and are then always rounded in form. The cytoplasm of the amœbocytes is more or less granular like that of the bottom cells of the septal sacs and the neoblasts. The nuclei of the amœbocytes are not always nucleolated.

"Chloragogue granules," says RICE in his paper on *Lumbricus herculeus*, "are found free in the cœlomic fluid. They are found imbedded in leucocyte bodies..... Lastly they are found in the muscular tissue of the outer body wall, dorsally and ventrally: on the dorsal aspect, for the most part as diffuse granular masses distributed throughout the circular muscular layer; on the ventral aspect in more compact masses in the vicinity of the setæ." Something similar may also be seen in *L. gotoi*, but I have grave doubt about the identity of the cœlomic and chloragogue granules. The ground for this doubt lies in the

fact, that the chloragogue granules stain hardly with eosin, while the cœlomic granules always come out well stained. The cœlomic granules are of unequal sizes and appear like coagulated yolk.

## 5. Nephridia.

The nephridia are present in pairs on the ventro-lateral side of the intestine, in all the segments except I–VI and IX–XII. The external orifices lie just in front of the ventral seta-bundles, and the funnel lies in the preceding segment. There are two forms of nephridia: one is represented by the nephridia of segments VII and VIII, and the other by those of the posterior segments beginning with XIII.

1). Anterior nephridia. "The mouth of the funnel is obliquely turned towards the median line and its margin is thickly covered with long cilia, which vibrate regularly in turn so as to make a wave pass from one end to the other. The cilia growing in the lumen of the canal are short and their

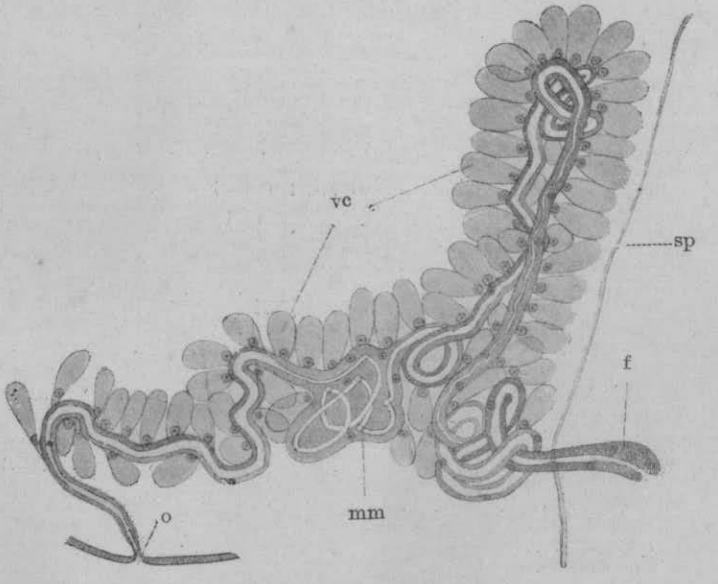



Fig. 7.

*L. gotoi.* Side view of anterior nephridium; reconstructed. ×150. f—funnel, mm—main mass, o—external orifice, sp—septum, vc—vesicular cells.

motion is very rapid" (HATAI). The funnel is continued into a long narrow canal which immediately pierces the septum. The canal undergoes a few convolutions and becoming smaller proceeds dorsalwards nearly parallel with the septum. At the dorsal end, it again becomes broader, undergoes two or three convolutions, and then proceeding ventralwards enters the main mass of the nephridium, in which the canal undergoes convolutions for the third time. After leaving the main mass the canal proceeds to the nephridial pore with a few windings on the way.

The chief characteristic of these organs is the constant presence of numerous large vesicular peritoneal cells attached around the nephridial canal, imparting a racemose appearance to the whole organ. These cells are club-shaped, and contain a thin cytoplasm. The nuclei are large, nucleolated and always situated at the proximal end of the cells.

2). Posterior nephridia. The funnel is exactly similar to that of the anterior nephridia. "The funnel is continued to a long narrow canal, which after undergoing manifold convolutions, enters the main mass of the nephridium" (HATAI). According to

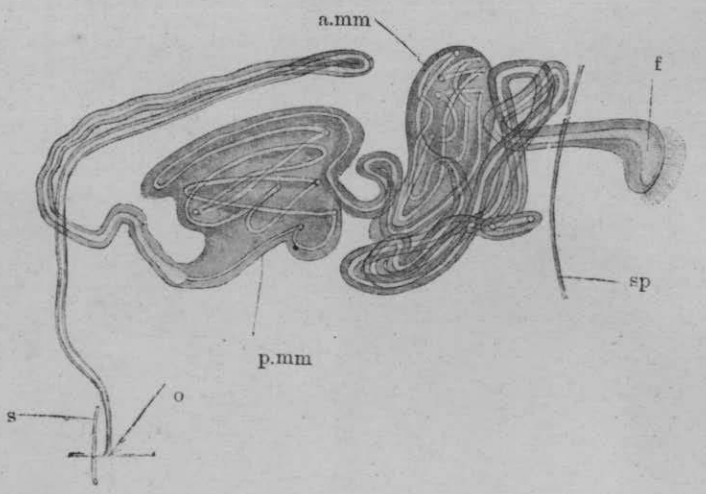



Fig. 8.

*L. gotoi.* Posterior nephridium from middle part of body; from a living specimen. ×150. f—funnel, sp—septum, a.mm—anterior main mass, p.mm—posterior main mass, o—external orifice, s—seta.

my own observations there are two main masses, an anterior and a posterior. The canal takes a long convoluted course in the anterior mass, and then leaves it for the posterior mass, in which it again undergoes manifold convolutions. On leaving the posterior mass, the canal becomes larger and finally opens to the exterior. In the posterior segments the course of the nephridial canal is simpler and the two masses are united into one. The club-shaped peritoneal cells are found very rarely, so that the organ never assumes a racemose appearance.

In each segment that contains the nephridia, numerous parieto-visceral muscle fibres run between the visceral organs and the body wall. They are attached to the circular muscle layer of the body wall at about the end of the first third of the segment and in the same longitudinal line with the nephridial funnel. The parieto-visceral muscle fibres are always united into bundles which diverge in several directions. Some of them are attached to the intestine, the ventral nerve cord and, in segment VIII, also to the heart, but the remaining bundles are attached to the nephridia at "the convoluted portion of the nephridial canal lying outside the main mass" (HATAI). In the anterior nephridia these muscular bundles are always attached to the dorsal end of the racemose portion.

The nephridial canal is intracellular throughout. The cilia are not visible in preserved materials, except in the funnel, but in life they are seen vibrating rapidly.

The nephridial cells have a more or less dense cytoplasm containing granules of different sorts. Some of them are exactly similar to those which are found in the cœlom. This fact was regarded by SCHNEIDER as proving the phagocytic action of the nephridial cells. These granules are also found in the peritoneal and seta-forming cells and often in the ganglion cells. These facts appear to me to throw some light upon the fate of the cœlomic granules.

The main masses of both forms of nephridia consist of peritoneal and nephridial cells mixed together.

The nephridial cells may be entirely destitute of a cell wall

or may be provided with it only on the outer surface. The nuclei are roundish and contain more than one nucleolus.

One or two nephridial ampullæ are sometimes present in the main mass, but they are inconstant in position. An end-vesicle may also be present; but they are in my opinion only temporary swellings of the canal.

HATAI says that, in *L. gotoi* the nephridia are present in segments VII–IX and XII to the last but one. But according to my own observations they are absent in segments IX–XII and in those that lie at a distance of two or more millimeters from the posterior end of the body; the latter point holds good for specimens which have not suffered any injury.

## 6. NERVOUS SYSTEM.

I can only give descriptions of the central system.

The brain lies on the dorsal side of the mouth cavity between the first and the second segment, and is held in its position in the cœlom by the longitudinal muscle fibres of the body wall. As seen from above it is almost square, somewhat narrowed anteriorly and shallowly notched on the posterior border. In a cross-section through its broadest part, it is kidney-shaped and convex dorsally, measuring 0.10–0.13 mm. across and 0.04–0.05 mm. dorso-ventrally. From the anterior corners of the brain two lateral prostomial nerves are given off on either side into the prostomium, in which they branch and subsequently enter the body wall. One of these branches becomes the lateral line already mentioned, which runs backwards to the hind end of the body. A median prostomial nerve starts from the anterior border of the brain, and is enlarged into a prostomial ganglion lying in the prostomial cavity, from which nerve fibres are given off in several directions. The median prostomial nerve is very slender, while the lateral ones are very stout. The prostomial ganglion is short spindle-shaped, 0.015–0.02 mm. long and 0.008–0.01 mm. broad.

The peripharyngeal commissures run out from the posterior corners of the lateral prostomial nerves. After giving off branches

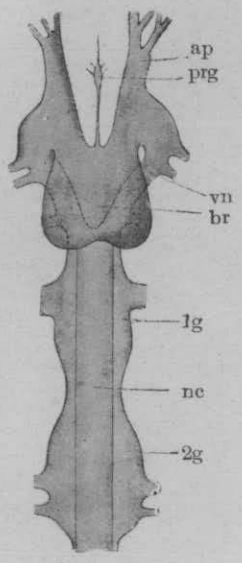



Fig. 9.

*L. gotoi.* Brain and anterior portion of ventral nerve cord; reconstructed. ×130. ap—lateral prostomial nerve, prg—prostomial ganglion, br—brain, vn—peripharyngeal commissure, nc—ventral nerve cord, 1g—first ventral or subpharyngeal ganglion, 2g—second ventral ganglion.

to the body wall, they proceed downwards and unite with the subpharyngeal ganglion at its anterior corners.

The subpharyngeal ganglion lies under the pharynx in segment II. The ventral ganglia are large, occupying nearly the whole length of the segments, and give off certain pairs of nerves to the body wall and the visceral organs. In the ventral nerve cord are found three longitudinal canals, one median and two lateral.

The median canal commences mostly in segment V, but in younger specimens it sometimes begins more posteriorly. In the anterior part of the body the canal is more spacious than in the posterior, and it is always situated at the dorsal median line of the nerve cord. The lateral canals commence in segment II; they are at first inconstant in position, but in segment IV they lie close to the dorsal border of the cord; and from segment V backwards they are either united or lie side by side with the median canal. These lateral canals are small in the anterior segments, but always larger than the median canal in the posterior segments. In the segments near the end of the body none of these canals are present. They contain a slightly staining substance and are bounded by nerve and muscle fibres.

The brain apparently consists of two layers: an outer layer consisting of ganglion cells with dense cytoplasm and an inner surrounded by the former dorsally and laterally. The inner layer is entirely made up of nerve fibrils which run in all directions and intermingle with one another. These two layers are present in all the other ganglia; but their relative positions are reversed in the ventral nerve cord, the cellular layer lying here on the

ventral side. The fibrillar portion, which is elliptical or rhomboidal in cross section, is bounded by special branches of the parieto-visceral muscles.

## 7. Vascular system.

This system consists of the dorsal vessel, the ventral vessel, the supra-intestinal vessel, the commissural vessels, and the contractile hearts.

1). The dorsal vessel runs through the whole body length on the dorsal side of the alimentary canal. It commences directly under the prostomial ganglion and runs straight backwards. Running directly under the brain, it passes through the posterior notch of the latter and comes to lie on a level with the upper surface of the brain (fig. 10). It then continues its backward course, and sometimes undergoes windings in the segments behind the fifth. It is to be noted especially that it shifts its position to the ventral side and lies near the ventral vessel in segments IX–? XVII, which contain the genital organs. Behind these segments it reassumes its position on the dorsal side.

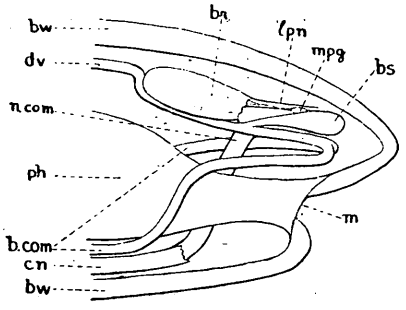



Fig. 10.

*L. gotoi.* Diagrammatic side view of head; lateral prostomial nerve, nerve-commissure and body wall of the right side omitted. br—brain, bw—body wall, b.com—commissural vessels, n.com—pharyngeal nerve-commissure, lpn—left lateral prostomial nerve, mpg—prostomial ganglion, bs—blood sac, dv—dorsal vessel, cn—ventral nerve cord, ph—pharynx, m—mouth.

2). The ventral vessel runs through the whole length of the body on the ventral side of the alimentary canal and mostly attached to it. It is paired in its anterior portion, which commences immediately under the pharynx at the middle of segment I, and the two vessels unite at the middle of segment V, where it receives a small median vessel coming from the anterior part of the alimentary canal (fig. 11).

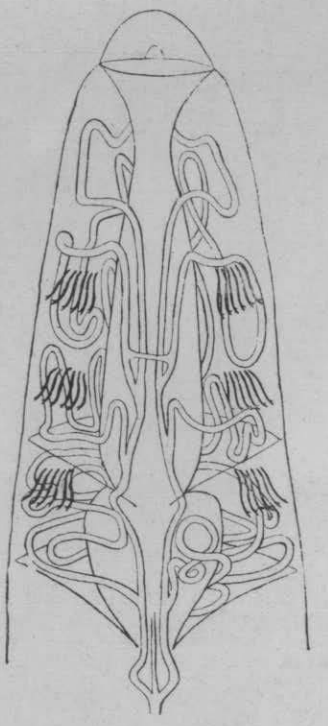


Fig. 11.

*L. gotoi.* Ventral view of the anterior end of the body, showing the blood vessels; reconstructed. ×100.


Fig. 12.

A portion of the intestinal vascular network. siv—supra-intestinal vessel.

3). The supra-intestinal vessel is always attached to the dorsal side of the intestine. It originates as a branch of the dorsal vessel in segment V, and opens again into the dorsal vessel at the posterior part of the body. A small vessel is given off from it anteriorly to the alimentary canal before it opens into the dorsal vessel in segment V.

4). Two forms of commissural vessels are found:

*a.* The intestinal networks connect the supra-intestinal with the ventral vessel. They are well developed in all the segments behind the fifth. These networks are connected with both the supra-intestinal and ventral vessels at the middle of a segment, there being sometimes several connecting vessels. In the posterior segments, they are connected with the dorsal instead of the supra-intestinal vessel; otherwise the arrangements are the same as in the anterior segments. In segments II–IV, the intestinal vascular networks are very weakly develeped.

*b.* One pair of commissural vessels of another sort lie in each segment. They have no direct connection with the alimentary system and may be subdivided into two categories: the cœlomic vessels and the integumentary vessels.

i). The cœlomic vessels are pre-

sent in all the anterior segments except VIII. These vessels enter into intimate relations with several internal organs, and must therefore be described separately for each segment.

The dorsal vessel is divided at its anterior end into two branches, which are the commissures of segment I. They run backwards on the dorsal side of the mouth cavity and after undergoing a few windings in the first segment, open into the anterior ends of the ventral vessels.

In segment II, the commissures are given off from the dorsal vessel at the posterior part of the segment. They proceed anteriorly to the middle of the first segment, and then, turning backwards and

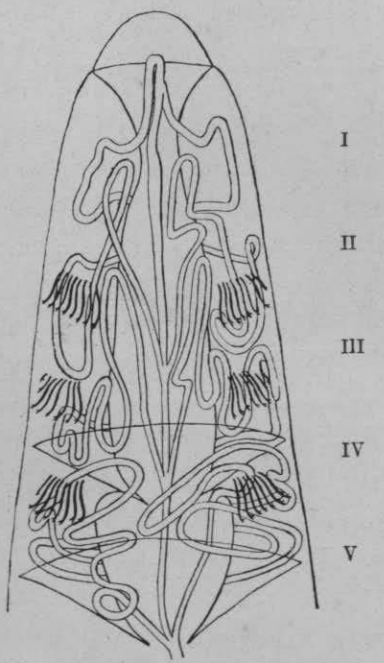



Fig. 13.

*L. gotoi.* Dorsal view of the anterior part of the body, showing the blood vessels; reconstructed. ×100.

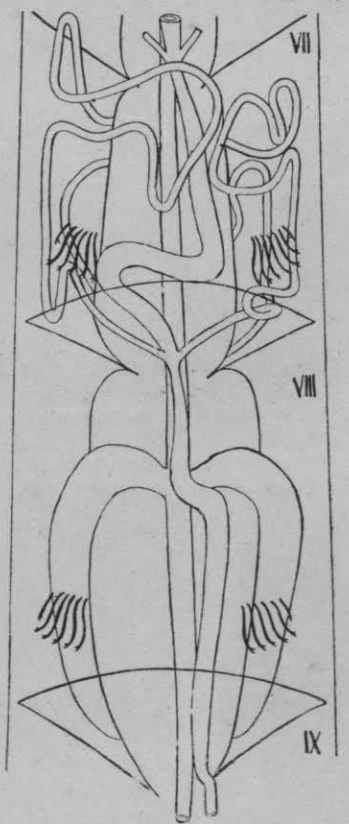



Fig. 14.

*L. gotoi.* Dorsal view of segments VII and VIII, showing the blood vessels; reconstructed. ×100.

undergoing a few windings on the way, and after coursing on the ventral side between the nerve cord and the body wall, open into the ventral vessels at the posterior part of the segment. A transverse commissure is often present between these commissural vessels near where they unite with the ventral vessels (fig. 11).

The above description applies to the commissures of segments III–VII; but behind the fourth segment they are confined to their own segments by reason of the presence of the septa, and in these segments the commissures invariably start and end near the posterior septum of the segment.

In segment VIII they are absent.

In segment IX they undergo several convolutions around the anterior sperm-sacs.

In segment X the vessels enter the posterior sperm-sac, and in fully mature specimens they also enter the anterior sperm-sacs.

In segment XI the vessels first surround the prostate, and then enter the ovisac.

In segments XII–? XVII, in which the ovisac lies, the vessels always surround it.

In the more posterior segments the commissures run simply from the dorsal to the ventral vessel.

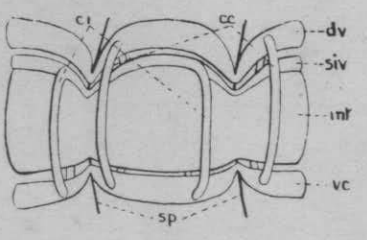



Fig. 15.

*L. gotoi.* Diagram showing the mutual connections of the blood vessels in the middle part of the body. dv—dorsal vessel, siv—supra-intestinal vessel, int—intestine, vc—ventral vessel, sp—septa, cc—cœlomic commissure, ci—intestinal network.

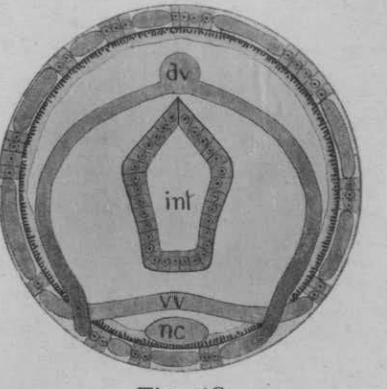



Fig. 16.

*L. gotoi.* Diagram showing the courses of the integumentary vessels in the posterior part of the body. dv—dorsal vessel, int—intestine, vv—ventral vessel, nc—ventral nerve cord.

ii). The integumentary vessels are always present in the posterior segments. They branch out from the dorsal vessel at the posterior part of a segment, run toward the ventral side, and immediately behind the ventral setæ they penetrate into the hypodermal layer of the body wall, in which they take an undulating course and come out again into the cœlom at the same point where they entered it, and then proceed straight to the ventral vessel into which they open.

In the posteriormost segments of the body these integumentary vessels are absent and the two or more commissural vessels form a large ring in the cœlom.

I have sometimes found a blood sac (fig. 10, bs) in both Japanese and Indian specimens. It lies on the dorsal side of the dorsal vessel in segment I, and is connected with the latter close to the origin of the prostomial nerves.

5). The contractile hearts lie in segment VIII, and originate from the supra-intestinal vessel between the middle and last third of the segment (fig. 14). They take a winding backward course on either side of the intestine, and on reaching the posterior septum, they make a sudden turn towards the ventral vessel and open into it. Sometimes they run parallel to and near the ventral vessel to the middle of segment IX, passing on the way through the septal sacs of VIII/IX.

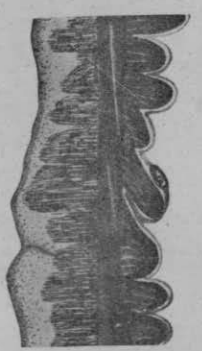


Fig. 17.

*L gotoi.* Part of the heart wall in cross section.

The wall of the contractile heart is very thick compared with that of the ordinary vessels. The outermost layer of the wall is the peritoneum, and inside of this lies a curious layer consisting of bundles of exceedingly fine, long fibres, which take up the fuchsin of MALLORY'S stain as well as eosin. They also stain deeply with HEIDENHAIN'S hæmatoxylin. I can not understand their nature. This layer is sometimes penetrated by intrusions of the peritoneal cells, which may possibly contain nerves.

Next to the above mentioned layer comes the circular muscle layer, which is well developed ;

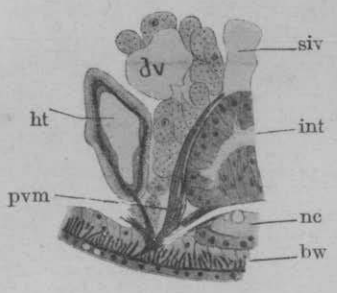



Fig. 18.

*L. gotoi.* Part of a cross section through segment VIII, showing the relation of the parieto-visceral muscles to the internal organs. ×150. dv—dorsal vessel, siv—supraintestinal vessel, ht—heart, int—intestine, nc—ventral nerve cord, bw—body wall, pvm—parieto-visceral muscle.

next lie the longitudinal muscle fibres united into bundles and last comes the intima, which lines the whole interior of the heart. The longitudinal muscle seems to act as valves at the ends of the hearts. The circular muscle layer of the heart is connected to the body wall by branches of the parieto-visceral muscles.

## 8. Genital system.

The organs which are to be described in this section are the testis, the sperm-sac, the sperm-duct, the spermatheca, the ovary, the ovisac, and the oviduct.

1). Testes. These are attached to the posterior face of septum IX/X and provided with a very thin membrane.

2). Sperm-sacs. In young specimens, in which the sperm-sacs have not been formed, the germ cells, liberated from the testes, aggregate on the dorsal side of the intestine and are found attached to the posterior face of septum IX/X. They are richly supplied with cœlomic commissural vessels in segment X. But after the formation of the sperm-sacs the sperm-cells are always found in them, and go through the stages of spermatogenesis there.

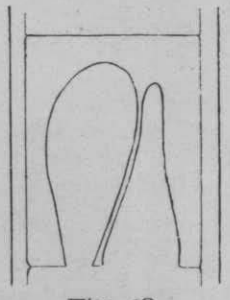


Fig. 19.

*L. gotoi.* Diagram showing an unequal development of the anterior sperm-sacs.

The sperm-sacs are outgrowths of the septa of the tenth segment. The anterior sperm-sacs originate in a pair from the anterior septum and lie in segment IX, while the posterior one is formed by the posterior septum and extends backwards. The posterior sperm-sac, when fully grown, traverses the entire length of segment XI, and is telescoped into the ovisac, sometimes

reaching to segment XVI. Sometimes the anterior sperm-sacs are unequally developed, but are never united.

The walls of the sperm-sacs are very thin and structureless, but are richly supplied with blood capillaries in mature specimens, either on the outer or inner side.

3). Sperm-ducts. Each sperm-duct consists of a wide funnel, a long narrow vas deferens, a spindle-shaped atrium with a glandular appendage, the 'prostate,' and a duct portion and a penis. The whole organ with the exception of the funnel lies in segment XI.

*a.* Funnel. The funnel is the internal opening of the sperm-duct; it is very wide and is attached to the anterior face of septum X/XI in segment X, on either side of the ventral median line. Its shape is somewhat urceolate, and the margin mostly shows irregular foldings.

The wall of the funnel consists of two layers. The inner layer consists of regularly arranged, ciliated columnar cells, measuring 10–12 $\mu$ by 4–5 $\mu$. They are very vesicular and contain in the distal part a small amount of feebly staining cytoplasm, which usually forms strands to support the layer against the pressure of the sperm. The cilia are all of nearly equal lengths, measuring 5–7 $\mu$. The nuclei, sometimes containing a single nucleolus, are in Indian specimens situated invariably in the proximal part of the cells, while in Japanese ones they lie invariably in the middle of the cells. This is the only point in which the two sets of specimens differ from each other. The outer layer of the funnel is formed by the connective tissue derived from the peritoneum.

*b.* Vas deferens. The funnel is continued into a very long, slender vas deferens, which undergoes several windings. It is circular in cross-section and of uniform diameter all through, viz. about 25 $\mu$. The lumen is about one third of the diameter, except for a short distance at the beginning, where it is wider. The tube is attached to the septum only at its beginning, while the remainder lies free in the cœlom, and consequently assumes several positions between other organs, and often extends into segment XII.

The wall of the duct consists of two layers, as in the case of the funnel. The ciliated cells of the inner layer are thin (2–3 $\mu$) and nearly semicircular in shape, so that two or three cells are sufficient to form the whole circumference of the canal. The plasmic strands are stouter than those of the funnel. The nuclei are exceedingly elongated, measuring 9–10 $\mu$ by 1.5–2.0 $\mu$, and lie in the middle of the cells, with their long axes perpendicular to that of the tube. The outer connective tissue layer is very thin.

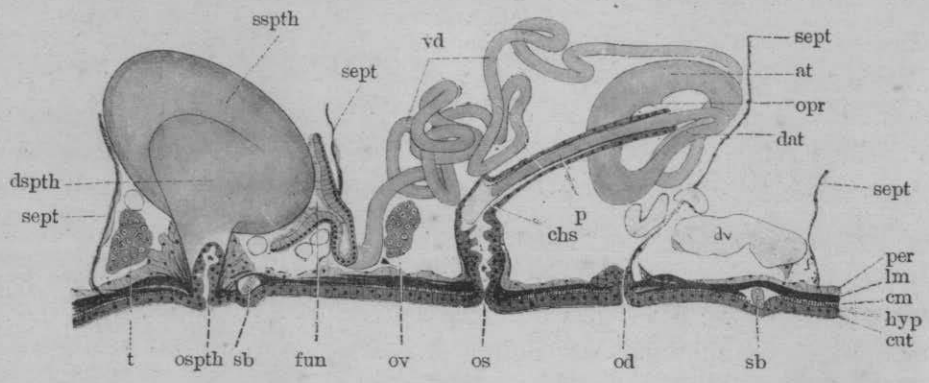



Fig. 20.

*L. gotoi.* Schematised longitudinal section through the left ventral setal line of segments X–XII, showing the mutual relations of the genital organs. Prostate gland omitted. The posterior end of the contorted spermatheca is situated directly in front of the sperm-duct funnel. sspth—saccular portion of spermatheca, dspth—duct-portion of spermatheca, ospth—spermathecal pore, sept—septum, t—testis, ov—ovary, sb—seta-bundle, fun—sperm-duct funnel, vd—vas deferens, at—atrium, opr—opening of prostate gland into atrium, dat—atrial duct-portion, p—penis, chs—chitinous penis sheath, os—male pore, od—oviduct, dv—dorsal blood vessel, per—peritoneum, lm—longitudinal muscle, cm—circular muscle, hyp—hypodermis, cut—cuticle.

*c.* Atrium. This is a spindle shaped organ which receives the vas deferens at one end and leads to its own duct portion at the other. The widest part of the organ lies at its middle and is more than three times the diameter of the vas deferens. It is also circular in cross-section.

The wall of the atrium consists of three layers. The outer layer is the continuation of the connective tissue of the vas deferens, and is followed throughout the organ by a compact muscle layer which appears to be circular but may possibly be spiral.

The innermost layer consists of glandular cells. The regularly arranged ciliated cells of the vas deferens become polygonal at its hind end like those of the funnel, and suddenly losing their cilia pass into the glandular epithelium of the atrium. The glandular cells are large and have a feebly staining cytoplasm containing numerous deeply staining secretory granules of various sizes; the nuclei always lie at the basal part of the cells during the period of active secretion. The cell membranes are exceedingly thin, and are difficult to make out. The inner cavity of the atrium is always irregularly crescent-shaped in cross section at its broadest part.

The process of secretion takes place as follows. The diffusely scattered secretory granules of the atrial gland cells first assemble in the distal part of the cells, in more or less distinct groups. The granules of these groups then unite and form large globules, which again unite to form large masses; these become entirely homogeneous and are finally expelled into the atrial cavity. As soon as the expulsion has been effected the cells resume their activity; and the next secretion is even proceeding in the proximal part of the cells before the expulsion of previous secretion has been completed.

*d.* Prostate. The prostate gland opens into the middle of the atrium, where the outer layers of the atrial wall are drawn out to receive the duct of the former, the two organs being separated by a sphincter muscle consisting of a few fibres. The organ is hemispherical in young specimens, but of irregular form in full grown ones, in which it surrounds the atrium more or less and occupies a large part of the cavity of the segment, sometimes pressing on septum XI/XII. The general aspect of the organ is well described by DIEFFENBACH in *Tubifex*. He says, "Sie besteht aus einzelnen länglichen Drüsenlappen, die von der Mündungsstelle aus sich fächerförmig ausbreiten und in deren Mitten ein feiner Kanal verläuft, dem die einzelnen, stark granulösen, mit grossem Kern und Kernkörperchen versehenen Drüsenzellen aufsitzen." The prolongations of the lobe canals, sometimes uniting with others, open into the interspace between the circular

muscle and the glandular cell layer of the atrium. Each lobe of the gland is surrounded by a thin connective tissue layer, which is a direct continuation of that of the atrium.

*e.* Duct portion of the atrium. The duct portion is about half as long as the atrium. On the side of the atrium, the canal is only 20 $\mu$ in diameter, and the inner epithelium becomes thinner and ceases to be glandular, the cytoplasm becoming compact and the cell membrane distinct. After a further diminution of its diameter, its epithelium is finally continued into the inner lining epithelium of the penis. The outer two layers—muscle and connective tissue—are the direct continuations of those of the atrium and of the same structure.

*f.* Penis and related portions. The penis is the direct prolongation of the duct portion of the atrium enclosed in a chitinous sheath and situated in the penial chamber, formed by the invagination of the body wall.

The wall of the penis consists of two layers. The epithelial cells of the inner layer contain very little cytoplasm adhering to the cell wall. The outer layer is a direct continuation of the lining epithelium of the penial chamber and is folded on itself at the proximal or posterior end of the penis. This layer gradually diminishes in thickness for some distance as it proceeds towards the distal end of the penis and is flattened out into an exceedingly thin membrane, but resumes its thickness and cellular condition toward the distal end, where the penis is slightly enlarged. The cells are perfectly similar in structure to those of the inner epithelium.

The chitinous sheath is attached to the hindmost end of the penial chamber, and measures about 35 $\mu$ in diameter. The sheath proceeds straight forwards with decreasing diameter (22 $\mu$); but near the distal end it again becomes thicker and after a slight curvature towards the median plane it terminates with a sudden expansion and deflection. The wall of the chitinous sheath is 2.3–2.5 $\mu$ thick, except at the deflected part where it is thinner.

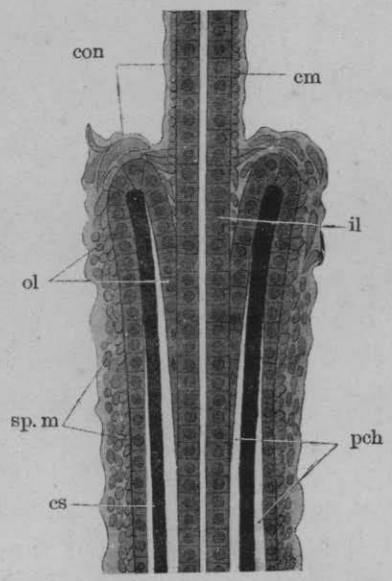



Fig. 21.

*L. gotoi.* Semidiagrammatic longitudinal section of penis and related organs; distal half not shown. con—peritoneal connective tissue, cm—circular muscle of atrial duct-portion, ol—lining epithelium of the penial chamber, il—inner epethelium of the penis, pch—penial chamber, sp.m—spiral muscle, cs—chitinous sheath.


Fig. 22.

*L. gotoi.* Penis; sketched by Dr. A. Willey from an Indian specimen. m—muscle fibres.

The lining epithelium of the penial chamber is attached to the chitinous sheath for a short distance at its base. It then becomes thinner very gradually and near the end of the penis it again thickens more or less suddenly and is finally continued into the hypodermis of the body wall. The male pores lie on either side of the ventral median line, behind the middle of segment XI, and in the same longitudinal lines with the ventral seta-bundles. The wall of the penial chamber near the external pore is irregularly folded and is composed of five layers, which are direct continuations of those of the body wall. The innermost layer is the cuticle; the next is the cellular hypodermis containing many gland cells, while the remaining layers are very weakly developed, the peritoneum being transformed into a thin connective tissue layer.

The spiral muscle fibres are attached to the body wall near the male pore, and are thickly developed at the penial portion. At the proximal end of the penis they are continued into the muscles of the atrium, and at the other end they are attached to the body wall at the posterior part of the segment, near the dorsal seta-bundle.

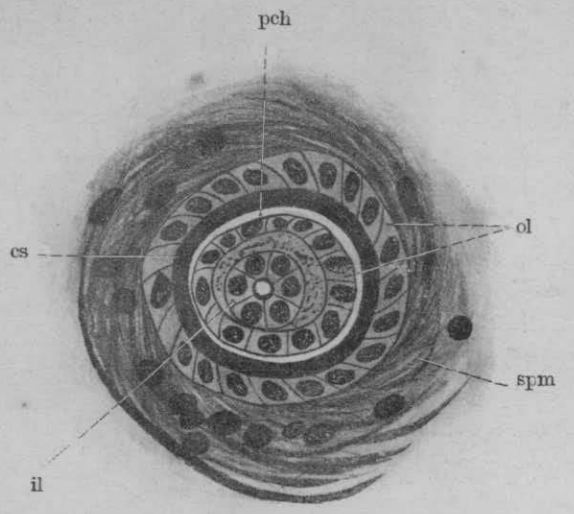



Fig. 23.

*L. gotoi.* Cross section through near the proximal end of the penis. ×700. pch—penial chamber, ol—lining epithelium of the penial chamber, spm—spiral muscle, il—inner epithelium of the penis, cs—chitinous sheath.

It is worthy of note that, in the most posterior part of the penial chamber, the cells of the two lining epithelia are obliquely disposed with respect to the long axis of the penis, owing to the action of the spiral fibres (fig. 23).

4). Spermathecæ. One pair of spermathecæ, formed by the invagination of the body wall, are present in segment X. When young the organ is a simple sac with thickened wall, but when fully developed it is somewhat pear-shaped, with a large saccular portion and a somewhat conical efferent duct.

The external opening lies in front of the ventral seta-bundle, at about the middle of the segment. In most cases the organs of either side undergo a sigmoidal contortion symmetrically with respect to the median line of the body, with the blind end directed anteriorly. In full grown specimens, however, the saccular portions are often situated directly under the dorsal median line with the blind ends turned in opposite directions, as shown in fig. 24.

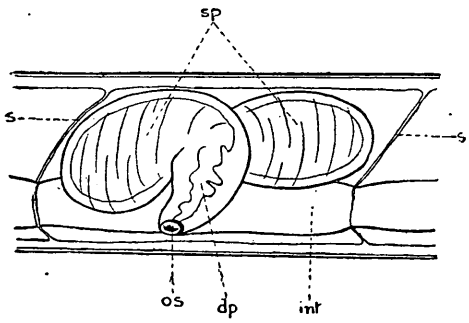



Fig. 24.

*L. gotoi.* Spermathecæ; from a living specimen, showing their asymmetrical position. sp—sacculär portions, s—septum, os—spermathecal pore, dp—duct portion, int—intestine.

The wall of the efferent duct consists of five layers, which are direct continuations of those of the body wall. The inner surface is lined by a thin cuticle, the next layer is cellular and folded irregularly, the cells having a dense cytoplasm striated perpendicularly to the axis of the duct; the circular muscle layer is well developed and the longitudinal also pretty well; the outer peritoneal epithelium is well developed, with club-shaped, nucleolated nuclei.

The three outer layers of the duct wall are continued with gradually decreasing thickness into those of the saccular portion, the cuticle also become exceedingly thin and totally imperceptible, so that the wall appears in this portion to consist of only two layers. The inner layer consists of large columnar gland cells, with a thin cell wall, a very large nucleolated nucleus and a compact cytoplasm containing numerous small secretory granules, especially in the distal half. This epithelium is followed by a connective tissue layer containing two feebly developed layers of muscular fibres: an outer one of longitudinal fibres and an inner of circular.

The spermathecal cavity is filled with fine granular contents, which are the secretions of the gland cells. The spermatophores are never present. VEJDOVSKÝ makes the same statement on *L. hoffmeisteri* CLAP., and the same fact has been ascertained in three tubificid genera *Hesperodrilus*, *Branchiura* and *Telmatodrilus*.

5). Ovaries. I have nothing to add to the statements of HATAI except that the ovary is provided with a thin membrane.

6). Ovisac. It is an outgrowth of septum XI/XII and extends posteriorly on the dorsal side of the intestine, sometimes

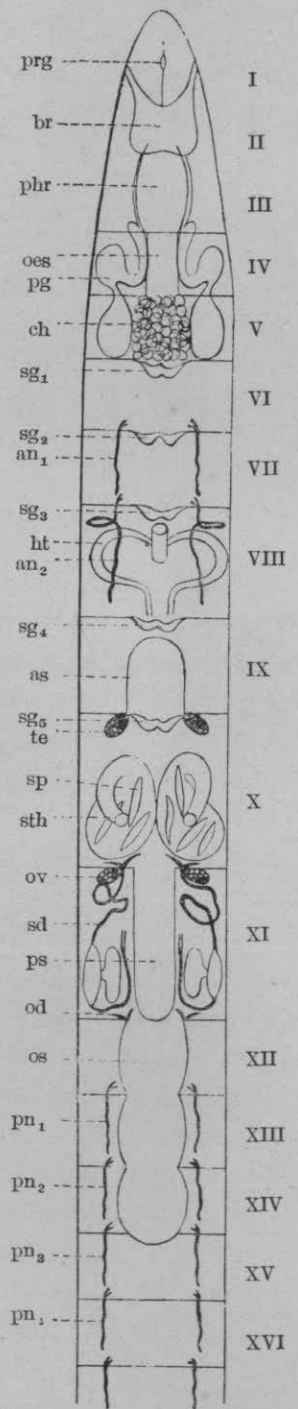
into segment XVII. Its minute structure is similar to that of the sperm-sac. In fully mature specimens it always contains eggs, which are going through the process of maturation and contain numerous yolk granules.

7). Oviducts. To the statements of HATAI it may be added that the oviducts lie in the same longitudinal line with the ventral seta-bundles. The hinder margin of the funnel is in contact with the anterior face of septum XI/XII. The cells of the wall of the oviduct are arranged radially around the external pore and always contain a compact cytoplasm.

## PART II. *Limnodrilus willeyi,* n. sp.

### 1. Description.

Colour of the body red, shaded posteriorly with light gray or yellow. In the posterior part of the body are seen very fine transverse striations due to septa, which are less numerous than in *L. gotoi.* Prostomium somewhat conical. Segments I–V always biannulate, anterior annulus longer. Brain almost square, emarginate posteriorly. Clitellum forming a complete ring, usually in segment XI, but sometimes occupying two segments and always including segment XI. Setæ sigmoid, furcate; branches curved; upper branch longer and larger than the lower. Each seta-bundle in the anterior segments consists of 4–6 setæ, in the middle part of 1–3, and in the posterior of 1–2. Five pairs of septal sacs on the ventral side of the intestine, attached to the posterior faces of septa V/VI–IX/X. Pharyngeal glands well developed. Pharynx in segment $\frac{1}{2}$ II and III. Œsophagus in segment IV. Intestine and chloragogues commence in segment V. The nephridia of segment VIII are sometimes racemose in appearance. Prostata spindle-shaped, about as large as the atrium. Chitinous penis sheath 3–4 times as long as its diameter at the proximal end; circular in cross section all through. Anterior sperm-sac in segment IX, unpaired. Posterior sperm-sac in segment XI only, unpaired. Spermathecæ in X, paired; spermatophores always present during the breeding season. Body




length 80–100 mm. Number of segments 100–180. Hab.—Morioka ; Tokyo ; Kagoshima.

## 2. Anatomy and Histology.

The minute structure of *L. willeyi* is closely similar to that of *L. gotoi*, but there are some differences between them, aside from the main characters of systematic importance above mentioned. Materials were killed, after narcotising with chloretone which is better than alcohol, in PERENYI's fluid. The cells are generally larger than in *L. gotoi*.

### 1. BODY WALL.

The body wall consists of five layers. Segments I–V are always biannulate, the anterior annulus being longer. A similar inequality of the two annuli is described by MICHAELSEN in *L. udekemianus*. The prostomium is conical and bluntly pointed.

The cuticle is thinner than in *L. gotoi*, being less than 1 $\mu$ thick, and covers the whole body.

---

Fig. 25.

*L. willeyi*. Diagram showing the principal organs. Behind segment V the intestine is omitted. prg—prostomial ganglion, br—brain, phr—pharynx, oes—œsophagus, pg—pharyngeal gland, ch—chloragogues, $sg_{1-5}$—septal sacs, $an_{1,2}$—anterior nephridia, ht—heart, as—anterior sperm-sac, te—testis, sp—spermatophore, sth—spermatheca, ov—ovary, sd—sperm-duct, ps—posterior sperm-sac, od—oviduct, os—ovisac, $pn_{1-4}$—posterior nephridia.

The hypodermis consists of regularly arranged columnar cells, with distinct cell-boundaries and basally situated nuclei. Some gland cells are always present between them. In the clitellum also the hypodermal cells are strictly arranged in one layer; they are compressed and metamorphosed into fibrous supporting cells between the clitellar gland cells, which are larger and taller than the ordinary ones, being 30–33 $\mu$ high and 10–13 $\mu$ broad. The nuclei are irregular in shape and comparatively small, and lie near the circular muscle layer.

The circular muscle layer is well developed in the anterior part of the body, but becomes thinner in the posterior.

The longitudinal muscle layer is also well developed in the anterior part of the body and is divided by the lateral lines into two portions, the dorsal and the ventral.

The peritoneum of the body wall consists of irregularly shaped, large, vesicular cells. In segments IV–VI, it is very thick; but in the more posterior segments it is of the same thickness as the longitudinal muscle layer and usually do not project into the body cavity. The nuclei are large and nucleolated, and mostly lie near the inner side of the layer.

2. SETÆ AND SETA-BUNDLES.

Fig. 26.
*L. willeyi.* A seta.

The sigmoid setæ are nodulate and furcate at the distal end. The branches appear unequal in length even with a low power; the upper branch being always longer and stronger than the lower, and both strongly curved. The node is somewhat ridged at the middle. The setæ always form bundles, which are arranged in longitudinal rows and are present in all the segments except the first. In the biannulate anterior segments, the seta-bundles always lie in the longer anterior annuli; but in most segments they are generally situated in the posterior half. In segment XI, the ventral seta-bundles are absent.

The minute structure of the setigerous organs is the same as in *L. gotoi.*

### 3. Alimentary system.

The mouth lies on the ventral side of segment I, and is continued directly into the buccal cavity. The latter is flattened dorso-ventrally and opens into the pharynx at the middle of segment II, with a constriction between them.

The pharynx reaches to the end of segment III. It is well differentiated by the development of the pharyngeal glands. It is semicircular in cross-section; the lumen is concave ventrally and there is no median inlet. The wall of the pharynx consists of two layers: an inner of long cylindrical, ciliated, endodermal cells, forming a pad-like projection into the pharyngeal cavity on the ventral side, and a thin outer of connective tissue containing feebly developed blood vessels and pretty well developed muscle fibres.

The pharyngeal glands are arranged in six longitudinal rows on the dorsal side of the pharynx, beginning directly behind the brain and extending to the middle of segment III, with the exception of the two lateral or outermost ones, which run back into segment V; but all open into the pharynx in segment III. Each lateral gland presents a dorsal swelling in segment IV and is considerably enlarged at its hind end in

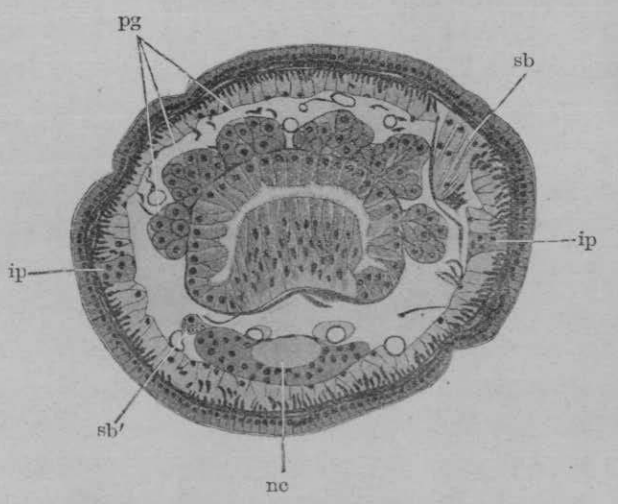



Fig. 27.

*L. willeyi.* Cross section through segment III, to show the pharyngeal glands. ×150. ip—lateral line, sb—setigerous organ, sb'—cell of young setigerous follicle, nc—ventral nerve cord, pg—pharyngeal glands.

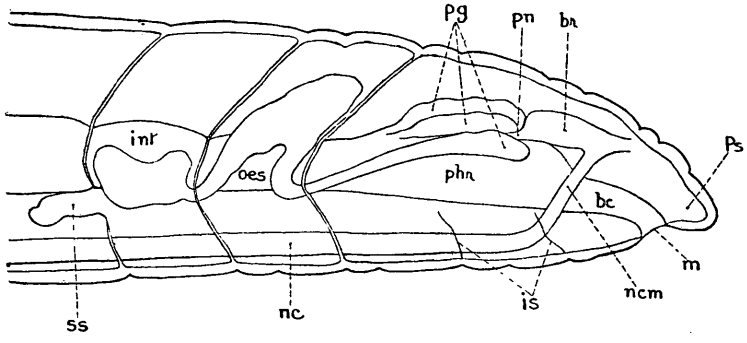



Fig. 28.

*L. willeyi.* Diagram to show the form and position of the pharyngeal glands. ps—prostomium, m—mouth, bc—buccal cavity, phr—pharynx, oes—œsophagus, int—intestine, br—brain, pg—pharyngeal glands, ncm—peripharyngeal nerve commissure, nc—ventral nerve cord, ss—septal sac, is—incomplete ventral septa, pn—pharyngeal nerve.

segment V, where it lies on the ventro-lateral side of the intestine. Each gland consists of a compact mass of pear-shaped cells about 20 $\mu$ long, enveloped by a thin layer of connective tissue. The cytoplasm of the gland cells stains feebly but contains numerous secretory granules, which take up DELAFIELD'S hæmatoxylin well. A large nucleolated nucleus lies in the middle of each cell. The proximal end of the gland cell is prolonged to form its own duct, which either unites with others coming from neighbouring cells or opens directly into the pharyngeal cavity. The efferent ducts from the parts of the lateral glands lying in segments IV and V, proceed anteriorly and open at the middle of segment III, in the same transverse line with the other rows.

The œsophagus is narrow and is confined to segment IV; its inner cavity is flattened dorso-ventrally. The ciliated endodermal cells contain each a nucleus at the middle. A few chloragogue cells are always present on the dorsal side of the œsophagus.

The intestine commences in segment V and terminates with the anus, which is dorsal in position. The inner epithelium is ciliated; the vascular layer which comes next is well developed and is surrounded by feebly developed muscle fibres. The chloragogue layer is very well developed in the anterior portion of the body, especially in segments V–VIII. Each chloragogue cell

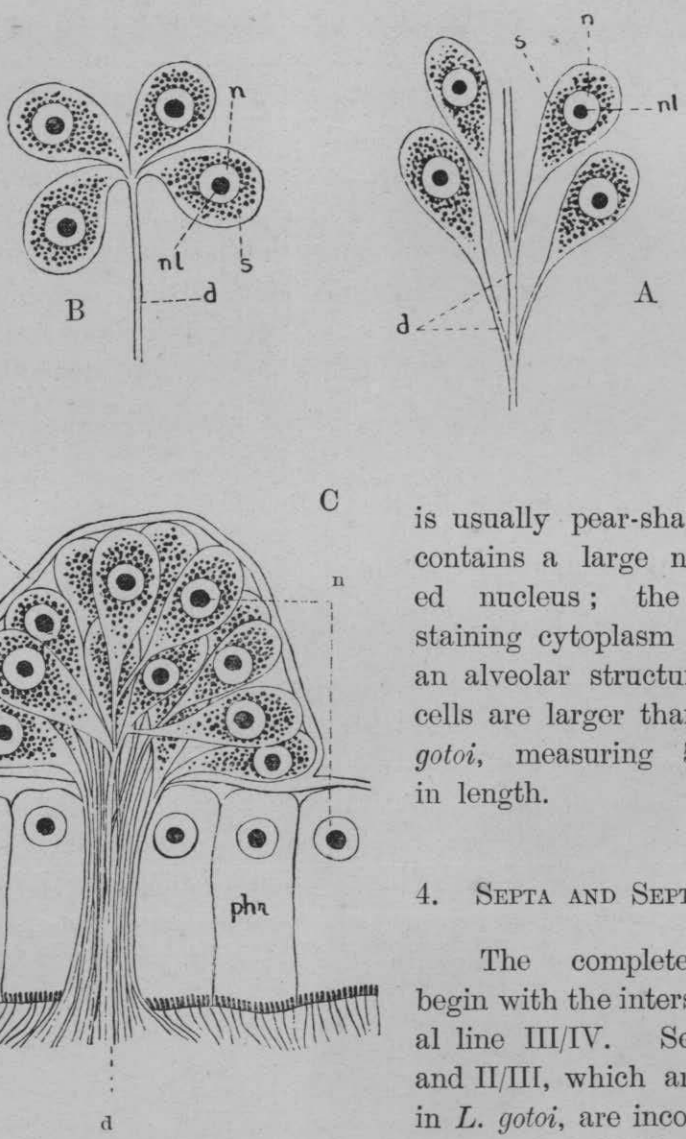



Fig. 29.

*L. willeyi.* Pharyngeal glands. A, B—from segment IV and V. C—a whole gland in cross section, from segment III. d—duct, n—nucleus, s—secretions, nl—nucleolus, phr—endodermal cells of the pharynx, ct—peritoneal connective tissue, bl—vascular layer of the pharynx.

is usually pear-shaped and contains a large nucleolated nucleus; the feebly staining cytoplasm presents an alveolar structure. The cells are larger than in *L. gotoi*, measuring 50–100 $\mu$ in length.

### 4. Septa and Septal sacs.

The complete septa begin with the intersegmental line III/IV. Septa I/II and II/III, which are absent in *L. gotoi*, are incompletely developed in this species, being present only on the ventral side of the alimentary canal.

The septal sacs lie on the ventral side of the intestine and are attached to

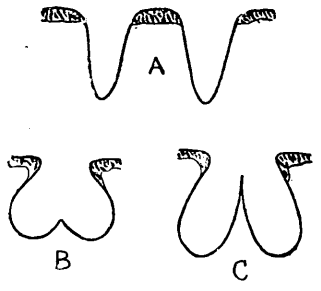



Fig. 30.

Diagrams showing the shapes of the septal sacs; A—*L. gotoi*; B, C—*L. willeyi*.

the posterior faces of septa V/VI–IX/X. They are of an ovoidal shape. In *L. gotoi* the sacs of a pair open separately into the cœlom, but in the present species the two sacs unite partially and form a common opening in the ventral median line of the body. Each sac must therefore be described as lying in the median line and divided into two at the hind end.

## 5. Nephridia.

The nephridia are present in pairs in all the segments except I–VI, IX–XII and those of the posterior part of the body. All have the same structure, opening internally in a segment and externally just in front of the ventral seta-bundles of the next following segment. Hatai's description of these organs in *L. gotoi* refers in reality to those of the present species. According to my own observations, the presence of an ampulla and an end-vesicle appears to be inconstant. The nephridial canal lies in the main mass, which consists of nephridial and peritoneal cells, but there are no ovoidal cells that give rise to the racemose appearance in *L. gotoi*. In segment VIII, these cells are often present, but they are only few and scattered.

## 6. Nervous system.

The brain lies in segment I, on the dorsal side of the buccal cavity. As seen from above it is almost square and shallowly notched posteriorly. A pharyngeal nerve (fig. 28, pn) runs out from each posterior corner of the brain, on the dorsal side of the pharynx, and enters the latter at the very commencement of the pharyngeal glands, between the lateral and outer dorsal glands. From the anterior part of the brain run out two strong lateral

and one slender prostomial nerves; the lateral lines branch out from the lateral prostomial nerves, while the median nerve leads to the prostomial ganglion, which is nearly twice as large as in *L. gotoi.*

The peripharyngeal commissures commence at the posterior corners of the lateral prostomial nerves and unite with the first ventral or subpharyngeal ganglion in segment II.

In the ventral nerve cord, the median neural canal begins in the middle of segment IV and the two lateral canals in segment V; they always lie side by side close to the dorsal border of the cord, with the middle one in the median line. The median canal is absent in the most posterior segments.

## 7. Genital system.

1). Testes. One pair of testes are attached to the posterior face of septum IX/X, close to the ventral nerve cord. Each testis is pear-shaped and is enclosed in a very thin membrane which is open at the dorso-posterior side.

2). Sperm-sacs. In the present species both the anterior and the posterior sperm-sacs are unpaired. The anterior one, lying in segment IX, is an anteriorly directed outgrowth of the anterior septum of segment X, and lies on the dorsal side of the intestine. It is kidney-shaped and large and contains numerous sperm cells in different stages of development. The posterior one is a posteriorly directed evagination of the posterior septum of segment X and is confined to segment XI. It lies on the dorsal side of the intestine, although its blind end sometimes tends to lie more ventrally, and is less well developed than the anterior sperm-sac. The sacs have the same structure as in *L. gotoi.*

In *L. gotoi*, only small numbers of sperm cells are present in the cœlomic cavity of segment X, but in this species, they are very numerous and richly supplied with cœlomic vessels.

3). Sperm-ducts. These lie in segment XI.

*a.* Funnel. It is very wide and attached to the anterior face of septum X/XI, on each side of the ventral median line.

Its wall consists of two layers, an inner of regularly arranged, ciliated, columnar cells and an outer of connective tissue. The nuclei of the inner epithelium is situated basally, and the plasmic strands are very feebly developed.

*b.* Vas deferens. The funnel is continued into a very long narrow vas deferens, which undergoes several windings. It is circular in cross-section and is of the same calibre through its whole length. The structure of the wall is nearly the same as in *L. gotoi*, except that the plasmic strands are very numerous and compact, and the nuclei less elongated.

*c.* Atrium. It is the enlarged portion of the sperm-duct and receives the vas deferens at one end. Its wall consists of three layers: an outer of connective tissue, a middle of muscle fibres, and an inner of large glandular cells.

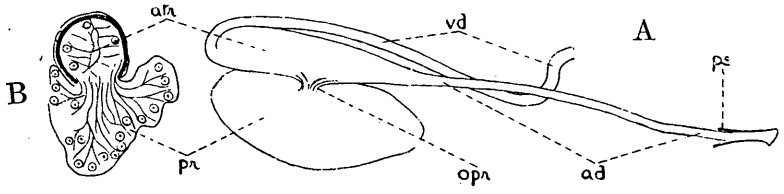



Fig. 31.

*L. willeyi.* Prostate gland and related portions. A—from a living specimen. B—cross section through the opening of the prostate. atr—atrium, ad—atrial duct-portion, opr— opening of the prostate gland into the atrium, pr—prostate gland, vd—vas deferens.

*d.* Duct portion. The duct portion is longer than the atrium proper, and is of the same structure as in *L. gotoi* except that the inner layer is ciliated.

*e.* Prostate. The prostate gland is somewhat spindle shaped and opens widely into the broadest part of the atrium. Its minute structure is the same as in *L. gotoi.*

*f.* Penis. It is enclosed by a chitinous sheath and projects into the penial chamber, formed by the invagination of the body wall. The wall of the penis consists of two layers, the outer of which is a direct continuation of the lining epithelium of the penial chamber, and the inner, which is not ciliated, of the inner

wall of the duct portion of the atrium. The chitinous sheath is nearly straight and is expanded like a trumpet at its distal end.

Fig. 32.

*L. willeyi.* Chitinous penis sheath.

The penial chamber opens externally on each side of the ventral median line at about the middle of segment XI. The muscle fibres of the outermost layer of the wall of the penial chamber run parallel to the axis of the penis and not spirally as in *L. gotoi.*

4). Spermathecæ. One pair of sigmoidal spermathecæ open at the anterior part of segment X in front of the ventral seta-bundles. The saccular portion is separated from the duct by a constriction and lies generally in its own side of the body, but sometimes the spermathecæ are decussated (fig. 34). The minute structure is

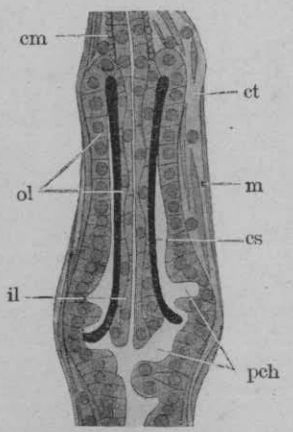



Fig. 33.

*L. willeyi.* Penis and penial chamber in longitudinal section. cm—circular muscle of the duct-portion of the atrium, ct—peritoneal connective tissue, m—longitudinal muscle fibre of the penis, ol—lining epithelium of the penial chamber, il—inner wall of the penis, cs—chitinous sheath, pch—penial chamber.

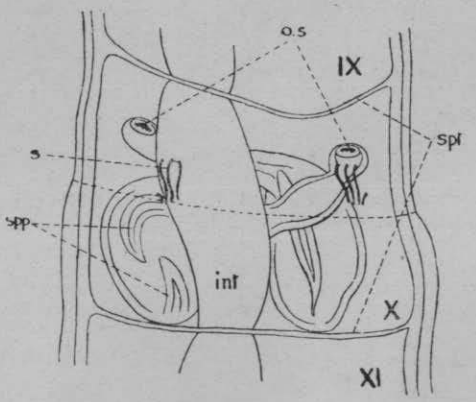



Fig. 34.

*L. willeyi.* Spermathecæ and neighbouring organs, from a slightly compressed living specimen. os—spermathecal pores, spt—septa, s—setæ, spp—spermatophores, int—intestine, IX, X, XI—numbers of segments. The anterior end of the clitellum is shown by a broken line at the middle of segment X.

the same as in *L. gotoi.* Spermatophores, which are mostly spindle shaped, are always present in the saccular portion.

5). Ovaries. One pair of ovaries lie attached to the posterior face of septum X/XI, close to the nerve cord.

6). Ovisac. It is an outgrowth of septum XI/XII and extends posteriorly sometimes into segment XVII, on the dorsal side of the intestine.

7). Oviducts. One pair of oviducts lie in the intersegmental line XI/XII, in the same longitudinal line with the male openings. The funnel of each duct opens widely into the cœlomic cavity of segment XI and the duct is very short.

### 8. Vascular system.

The arrangement of the principal vessels is the same as in *L. gotoi*. One pair of contractile hearts lie in segment VIII. As to the commissural vessels, it is to be noted that those of segment X are very well developed, and undergo convolutions in the cœlomic cavity, in which float numerous sperm-cells. Blood corpuscles are very numerous.

## Comparison of *L. gotoi* and *L. willeyi*.

The more prominent differences of the two species treated of in this paper may be shown in a tabular form, as follows.

| *L. gotoi*. | *L. willeyi*. |
|---|---|
| *The anterior segments are divided into two annuli, a shorter anterior and a longer, seta-bearing posterior. | *The anterior segments are divided into two annuli, a longer seta-bearing anterior and a shorter posterior. |
| *The branches of a seta are of nearly equal length. | *The branches of a seta are of unequal lengths. |
| The cell boundaries of the hypodermis are absent. | The cell boundaries of the hypodermis are distinctly present. |
| The pharyngeal glands are very feebly developed. | The pharyngeal glands are very well developed. |
| The chloragogue cells measure 35–50 $\mu$ long. | The chloragogue cells measure 50-100 $\mu$ long. |
| *The anterior nephridia are of racemose appearance. | *The anterior nephridia are not of racemose appearance. |

| | |
|---|---|
| There are no pharyngeal nerves. | One pair of strong pharyngeal nerves run out from the posterior corners of the brain. |
| The anterior sperm-sacs are paired. | The anterior sperm-sac is unpaired. |
| The posterior sperm-sac reaches sometimes to segment XVI. | The posterior sperm-sac is confined to segment XI. |
| The prostate opens narrowly into the atrium. | The prostate opens widely into the atrium. |
| The duct portion of the atrium is about half as long as the atrium, and its inner epithelium is not ciliated. | The duct portion of the atrium is longer than the atrium, and its inner epithelium is ciliated. |
| The wall of the penis is incompletely two layered. | The wall of the penis consists of two layers throughout. |
| *The chitinous penis sheath is 10–11 times as long as its diameter at the proximal end. | *The chitinous penis sheath is 3–4 times as long as its diameter at the proximal end. |
| The muscle fibres surrounding the male copulatory apparatus are spiral. | The muscle fibres surrounding the male copulatory apparatus are straight and parallel to the long axis of the penis. |
| *Spermatophores are not present. | *Spermatophores are always present in the spermathecæ. |
| *The prostate is of irregular shape. | *The prostate is spindle shaped. |
| No septa in the intersegmental lines I/II and II/III. | Septa I/II and II/III incomplete, being present only on the ventral side of the intestine. |
| The two septal sacs of a septum open separately. | The two septal sacs of a septum have a common opening. |
| The prostomial ganglion is 0.015–0.02 mm. long and 0.008-0.01 mm. broad. | The prostomial ganglion is nearly twice as large as in *L. gotoi*. |
| Blood corpuscles are very few or entirely wanting. | Blood corpuscles are very numerous. |

Characters marked with * are easily observable in the living state.

Comparing these two Japanese species with the European and American, as described or diagnosed by EISEN, VEJDOVSKÝ,

VAILLANT, BEDDARD and MICHAELSEN, the following may be noted: —

1. *L. hoffmeisteri* CLAP., *L. dugèsi* RYBKA and *L. igneus* (EISEN) resemble *L. gotoi*.

2. *L. udekemianus* CLAP. resembles *L. willeyi*.

In this paper I can not however enter into a detailed comparison of these forms, and although the resemblances between *L. gotoi* and *L. hoffmeisteri*, and between *L. willeyi* and *L. udekemianus* are very close, their discussion must be postponed.

December 20, 1912.

## Postscript.

While this paper was in preparation for the press my attention was called to two recent papers by Prof. STEPHENSON, excerpts from which were kindly sent to me by Prof. GOTO. They deal among others with a species of *Limnodrilus* which the author calls *socialis* and which he found in Lahore and Kandy, India. A perusal of the descriptions has convinced me that STEPHENSON'S *L. socials* is identical with *L. gotoi*, though there are some apparent differences between his descriptions and mine, some organs being called by different names. I must therefore go into a brief discussion of these differences in this postscript.

The septal sacs, which STEPHENSON regards as appendages of the alimentary canal, are in general of about the same size and shape in the two forms, although in *L. gotoi*—both in the Ceylon specimens collected by Prof. WILLEY in a temporary stream in Buller's Road, Colombo, on January 16, 1910, and in the Tokyo specimens collected by myself in summer—, so far as my observations go, there are no septal sacs in segment V and those of segment VIII are not notably smaller than those of segments VI and VII, as STEPHENSON says. Moreover his figure (Trans. Roy. Soc. Edinburgh, 48, pt. II, 1912, fig. 11) shows the septal sacs to be larger and more elongated dorso-ventrally

than in *L. gotoi.* These differences in the development of these organs may perhaps furnish a clue to their function; for it seems to me that they are larger during the active season of the animal. Locality and climate or temperature are also factors to be considered; for in none of the Tokyo specimens of this species have I observed septal sacs of such size as is figured by STEPHENSON, although some of these specimens were larger than any from Ceylon. At any rate, it is interesting to observe that the condition of the septal sacs in the summer specimens of Tokyo is the same as in the winter ones from Ceylon. It may be that *L. socialis* STEPHENSON represents older specimens of *L. gotoi*, which are either scarce or not found in Tokyo.

As to the sinus-like blood space especially described by STEPHENSON as being present in segment IX, I am of opinion that it must be inconstant, for a reëxamination of my sections shows it to be absent.

March 16, 1913.

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