# On Diplozoon nipponicum, n. sp.[1]

by

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With Plates XXI—XXIII.

Since *Diplozoon paradoxum* was first discovered and described by v. Nordmann,[2] it has been made the object of special investigations by many eminent naturalists. But our knowledge of the anatomy and especially the histology of this interesting génus, hitherto with but a single species, is, notwithstanding the publications of Paulson, Zeller, and others, by no means as complete as could be desired. I have, therefore, undertaken, at the suggestion of Prof. Ijima, to subject it to a renewed investigation. I at first believed that the Japanese species was identical with the European; but as I went on with my work, many points came to view, that made me doubt this identity; and a close comparison with some preparations of the European species taken from *Leuciscus rutilus*, and brought back from Germany by Prof. Ijima, has led me to erect it into a new species, for which I propose the name of

## Diplozoon nipponicum.

Before proceeding any farther, I must here discharge the pleasant duty of acknowledging my deepest obligations to

1) This paper was originally presented as a graduating dissertation.

2) Nordmann—Mikrographische Beiträge. I. Heft. 1832. p. 56.

Professor Ijima, already named, not only for constant supervision of my work, but also for lending me his books and preparations pertaining to the subject at hand. He has also handed over to me his unfinished manuscript, in which the anatomy and external features of many ectoparasitic Trematodes have been made out to a great extent —a circumstance for which I here express my warmest thanks.

*Dipl. nipponicum* is very common on the gill of *Carassius vulgaris*. Its differential characters as compared with *Dipl. paradoxum* are 1) the smallness of the posterior suckers, 2) the greater length of the posterior half of the body, 3) the shortness of the "connecting canal" between the intestine and the oviduct, 4) the presence of a pair of glands at the entrance of the mouth, and 5) the fact that the intestin, does not present lateral branches in the posterior portion of the body.

"Comment la réunion des Vers, a-t-elle lieu? sont-ils réunis comme les frères Siamois, ou bien sont-ils croisés comme les deux jambes d'un X?" By the investigations of v. Siebold[1] and Zeller,[2] it has been established beyond doubt that the double animal results by the union of two *Diporpae* in the form of a cross—a fact which had already been anticipated by Dujardin[3] their discoverer. The manner in which the two individuals are united, and the details thereof have already been made out by Zeller, who has also discussed the various opinions of his predecessors, and corrected their errors. I shall however, add a few remarks on some points not noticed by him, some of which are perhaps peculiar to the new species. For examining the external features of the worm, as well as for other purposes, it is best to kill it with boiling sublimate, in a watch-glass in which just sufficient water has been placed to cover its body. The worm which

1) v. Siebold—Ueber die Conjugation des Dipl. paradoxum. Ztschr. f. wiss. Zool. Bd. III. 1851. p. 62.

2) Zeller—Untersuch. ü. d. Entwicklung d. Dipl. paradoxum. Ztschr. f. wiss. Zool. Bd. XXII. 1872. p. 168.

3) Dujardin—Histoire naturelle des helminthes. 1845. p. 316.

has been killed in this way, preserves a natural position corresponding to its condition of rest, and can be examined when convenient.

Each individual, if considered separately, is elongated and lanceolate in form, with a deep notch on one side a little posterior to the middle of its whole length, by means of which it is united with the other individual ; so that we may hereafter speak of the anterior and posterior halves of the body. The anterior half is widest near the place of union, and becomes narrower anteriorly, where it ends with a rounded outline, and where the mouth is situated on the ventral side. In cross-section it presents an oval outline, which gradually becomes more circular as we proceed anteriorly. If the worm has died in a contracted state, the surface of the body is thrown into numerous transverse folds ; otherwise the surface is entirely smooth, except where little conical elevations, hereafter to be described, exist. The posterior half may briefly be described as an elliptic cylinder in the anterior portion, which, posteriorly, passes irregularly into a rectangular prism. It is also much slenderer than the anterior half. Seen in profile, the surface of the posterior portion is always, in specimens killed with hot sublimate, thrown into a number of strong folds, due no doubt to the powerful development of the diagonal muscular fibres in this region ; so that here the margin is deeply crenate or even zig-zag (Pl. XXI, Fig. 1). A cross-section through one of the posterior zig-zag folds presents nearly a rectangular outline. The folds suddenly come to a close at a short distance before the beginning of the posterior suckers. In this portion the cross-section presents a flattened ellipse ; and this part is, on surface view, distinctly marked off from the sucker-bearing portion which directly follows it, and still more so from the strongly folded more anterior portion, by a sudden change of level (Fig. 1). The lateral margins of this sucker-bearing portion are suddenly thickened on the ventral side, so that a cross-section through it is somewhat ⊔⊔-shaped. Under the pressure of a cover-glass, this portion assumes a somewhat oval form—a circumstance which probably induced v. Nordmann[1] and Paulson[2] to indicate it as an oval "Scheibe." Van Beneden[3] further speaks of "un pédicule" by which the "deux organes", which carry the suckers, are attached to the body; but I have not observed any such structure in my species, and it is probably due to a deformation caused by pressure and the extreme mobility of this part. The posterior margin of the body shows either a nearly straight line or, more commonly, a slight concavity (Fig. 2).[4] Toward this concavity, the body again becomes thicker, the thickening beginning this time in the median line, and thence spreading toward the sides, as indicated in Fig. 2, where the shading is made as if this portion were seen from the ventral side. On a longitudinal section, therefore, which does not pass through the lateral suckers, the body is seen to present posteriorly a club-like thickening, and end suddenly as if it were cut off. Van Beneden speaks of an "excavation plus ou moins profonde," by which he no doubt means the hollow, just spoken of, between the suckers.

The peculiar sudden bend (Knickung) towards the ventral side, which the body of the worm suffers at the place of crossing, has already been noticed by Zeller. There is, however, another feature not observed by him. If the worm, namely, is viewed from the posterior end, or if sections of this part are cut, it will easily be noticed that the bodies of the two individuals do not stand exactly opposite each

1) Nordmann—l. c. p. 60.

2) Paulson—Zur Anatomie v. Dipl. paradoxum. Mém. d. l'acad. St. Petersbourg. VII. Sér. T. IV. 1862. p. 4. I have not been able to gain access to this work, and am indebted for its account to Prof. Ijima's notes.

3) P. J. v. Beneden—Mémoire sur les vers intestinaux. p. 41.

4) The European species shows a decided convexity.

other, but that one is always a little either to the left or to the right side of the other, according as the worms are united by the corresponding sides of their bodies. This feature is usually less noticeable in the anterior halves, but it can easily be brought to view by bringing them close to each other. It is caused, no doubt, by the fact that the bodies of the two individuals are closely united *only* at the point of crossing; as may be seen, if one places two pieces of straw against each other in the form of a cross, and presses them together between two fingers at the point of crossing. Beside this imperfect apposition of the corresponding halves of the two individuals, must also be noticed the twist, to which each is subjected at the place of crossing, in consequence of the fact that one grasps with its ventral sucker the dorsal papilla of the other. To this twist, though very small in degree owing to the presence of the notch already mentioned, must be attributed the common occurrence that, when the worm is killed under the pressure of a cover-glass, the anterior and posterior halves of the same individual present to view opposite sides of the body—the anterior half presenting the dorsal if the other half presents the ventral side, and *vice versa*. The two individuals are united with each other by their sides, so that here a deep indentation arises—the notch already spoken of. Here the epidermis is absent, and the muscular layers of the two individuals are directly applied to each other. Zeller describes a direct connection between the vas deferens and "Laurer's canal" of the two individuals; but a careful examination has convinced me that this view is erroneous. I find Laurer's canal to open distinctly into the intestine, and the vas deferens of one individual into the yolk-duct of the other, as will be proved later on. In this connection, it may be mentioned that the same writer thinks the *Diporpa* incapable of "eine noch weiter gehende Entwicklung ohne dass zuvor die Copulation mit einer zweiten Diporpa zu Stande gekommen wäre."[1] But last summer I met with two *Diporpae* which were already producing eggs, but which were not united. They were attached to the same gill very near to each other. They were quite as large as any average *Diplozoon*, and measured about 6 mm. in length in a completely outstretched condition.[2] They were provided with four pairs of posterior suckers, but there was no trace either of the ventral sucker or of the dorsal papilla. In place of the ventral sucker, the longitudinal muscular layer was very strongly developed in the corresponding part; and the body shewed a sudden increase of breadth just anterior to the anterior end of the ovary, looking as if this part were bandaged. I have used the utmost care in detaching the worms from the gill, inasmuch as I carefully scraped off the gill-slime with a spatula, avoiding as much as possible any direct contact with the worms. The *Diporpae* in question were observed to be quite independent of each other from the moment they were detached from the gill; nor have I been able to detect any mechanical injury, or the notch by which they might have been united to each other; so that the chance of their being detached *Diplozoon* is, I believe, almost entirely excluded. Such a case of isolation is of course exceptional; but it shews that the *Diporpa* can, under certain conditions, become mature without uniting with another *Diporpa*.

It would have been interesting and instructive could I have determined where, in this abnormal case, the vas deferens opened. But unfortunately, owing to my inexperience then, I killed both the *Diporpae* under the pressure of a cover-glass and prepared them for gross mounting; and when I afterward cut one of them into sections,

1) Zeller—l. c. p. 176.

2) The size of the common Diporpa varies according to its stage of development. Dujardin gives it as 0.26—0.56 mm. in length and 0.18—0.35 mm. in breadth (l. c. p. 317). A specimen of the Diporpa of Dipl. parapoxum lent me by Prof. Ijima and possessing three pairs of suckers measured about 0.6 mm. in length. That of Dipl. nipponicum of the same stage is of about the same size.

I could no longer trace the course of such a delicate canal as the vas deferens.

Remark :—In Prof. Ijima's manuscript I find the following passage which I have his permission to publish.

"Ich will mir endlich noch eine Bemerkung über die von Heller[1] beschriebene Monstrosität erlauben. Dieser Forscher lässt, obschon ihm das Verhältniss des *Copulatio lateralis decussata* (Siebold) nicht fremd blieb, sein interessantes Exemplar sich dadurch erklären, dass die Verwachsung der beiden Diporpen sich über die ganze vordere Körperhälfte ausgedehnt hätte. Paulson, der sich übrigens mit die Ansicht Leuckart's theilt, dass die Diporpen einfach mit Bauchfläche zusammenhängen, hebt die Unmöglichkeit des Zustandekommens jener Monstrosität durch *Copulatio lateralis decussata* hervor, und nimmt an, es handele sich um eine Missbildung *per defectum* eines Diplozoons, bei welchem sich einer der Vorderleiber gar nicht entwickelt hätte. Dabei kam er sehr nah an die richtige Interpretation der Heller' schen Monstrosität, die meiner Ueberzeugung nach, nichts anderes sein kann, als eine Diporpa, nicht Diplozoon, mit in doppelter Anzahl angelegtem Schwanzende, also eine Missbildung *per adjectum*. Dies darf man nicht Wunder nehmen, denn wir wissen zahlreiche Fälle ähnlicher Missbildungen unter den Planarien. Ich kenne selbst einen Fall von ganz jungen, eben ausgeschlüpftem *Dendrocœlum lacteum* mit zwei hinteren Hälften, deren je eine einen Mund und einen Pharynx besitzt."

I shall now proceed to the consideration of the various parts.

1) Heller—Merkwürdiger Fall vorderer Verwachsung an Dipl. paradoxum. Sitzungsber. d. k. Akad. d. Wiss. Wien. 1857. p. 109.

## I. The Epidermis.

The nature of the integument of the Trematodes has been variously represented by various anthors. This subject I hope to discuss more fully in a later work which shall treat of our ecto-parasitic Trematodes in general. Zeller[1] tells us that if no occasion is offered the embryos to attach themselves to the gill, "schon nach Verfluss von 5 Stunden (after the embryos have left the egg) einzelne der Wimperzellen reissen sich los, bald mehrere und schliesslich alle, flimmern aber auch abgetrennt noch eine Zeit lang fort." The embryos finally die. It is not clear from his statements whether this throwing off of the "Wimperzellen" is a normal process or not. In *Polystomum* he merely says that they "schrumpfen," but does not describe their exact fate. In the case of *Distomum*, however, it has been proved by Schwarze[2] and Biehringer[3] that the so-called "cuticula" consists originally of cells which undergo one by one a peculiar transformation, and which do not at any time possess the typical epithelial arrangement. After the first rough manuscript of these pages had been finished, I received the article of Braun[4] in "Centrbl. für Bakteriologie u. Parasitenkunde," in which the writer brings forward some strong and interesting evidences as to the epidermal nature of the integument. In view of these facts established by the preceding investigators, I believe I may regard the integument of the monogenetic Trematodes as a modified epidermis,—the more so from the consideration that it has a distinct cuticle and

1) Zeller—l. c. p. 173.

2) Schwarze—Die postembryonale Entwicklung der Trematoden. Ztschr. f. w. Zool. Bd. XLII. 1886. p. 49.

3) Biehringer—Beiträge zur Anatomie u. Entwickl.-geschichte d. Trematoden. Arbeiten a. d. zool.-zoot. Inst. in Würzburg. Bd. VII. 1885. p. 4.

4) Braun, Max—Einige Bemerkungen ü. d. Körperbedeckung ectoparasitischer Trematoden. Centralbl. f. Bakteriol. u. Parasitenkunde. Bd. VII. 1890. p. 594 (Nr. 19.)

sits on a basement membrane. An embryological study, however, of the transformations which the original epidermis undergoes is, as Braun maintains, very desirable.

The integument of *Dipl. nipponicum* is composed of two layers, the cuticula and the underlying matrix. The cuticula, when examined in a living worm, is a very thin, structureless, refractive membrane. In sections of hardened specimens it appears as an insignificant line bounding the subcuticular (= epidermal) layer against the external world. It is very well seen in a living specimen which has been allowed to macerate in water for some time under the cover-glass. Numerous watery blisters then form in the epidermis, and separate the cuticula from the underlying layer. The former can then be examined as a separate structure. Transverse canals have been described in the cuticula of many Trematodes, but I have not observed any in the new species. The cuticula is reflected inward for some distance into the mouth.

Directly under the cuticula lies the epidermal layer, a uniform, granular matrix in which no nuclei are to be observed. I believe I have observed indistinct dark lines traversing the breadth of this layer but not quite reaching the cuticula. The epidermal layer, like the cuticula, is continued into the cavity of the mouth, and the sticky glands hereafter to be described (p. 166) are but local modifications of it. Wierzejski[1] describes the "Haut" of *Calicotyle Kroyeri* as consisting of "einer feinen Cuticularschicht mit den darunter liegenden kleinen, runden Matrixzellen"; but judging from his figure, I believe he has mistaken the nuclei of the connective tissue for his "Matrixzellen." The epidermal layer rests on a basement membrane, which eagerly takes up coloring matter, and is very conspicuous in cross-

1) Wierzejski—Zur Kenntniss des Baues von Calicotyle Kroyeri. Ztschr. f. wiss. Zool. Bd. XXIX. 1877. p. 552.

sections as a dark line with indistinct borders, separating the epidermal from the muscular layer. It is also much thicker than the cuticula. The total thickness of the integument, with the cuticula and basement membrane taken together, is about 0.004 mm.

It has already been mentioned that little conical elevations exist here and there on the surface of the body. These are more abundant on the ventral than on the dorsal side, and are entirely absent in the posterior half of the body. They are simple elevations of the epidermis with an almost homogeneous mass of connective tissue under it. Here the muscular layers do not touch the basement membrane, but pass straight on; so that these elevations are somewhat subject to changes of form. I have represented one of them in section in Fig. 7 (Pl. XXII). As will be seen, they are pointed at the end. A very similar structure has been described in *Sphyranura Osleri*,[1] where it seems to act as a sense organ. But although I directed my special attention to the point, and applied the highest magnifying power at my disposal (Zeiss Imm. L.), I could not discover any canal opening at the apex, or any hair-like projection, or any fibrils such as have been observed in the above-mentioned species to supply these conical bodies.

## II. The Muscular System.

The muscular system is constituted by the muscular wall of the body, the dorso-ventral muscles, and the muscles pertaining to the various organs.

The muscular layer of the body consists of three layers. These are, counted from outside inwards, the circular, the diagonal, and the longitudinal muscles. The circular fibres run everywhere immediately

1) R. Wright and Macallum—Sphyranura Osleri: a Contribution to American Helminthology. Journ. of Morph. Vol. I. 1887. p. 9.

beneath the basement membrane. They run isolated without forming bundles. This layer is most strongly developed at the anterior extremity of the body in the region of the anterior suckers, and immediately anterior to them, especially on the ventral side (Fig. 9), where its thickness amounts at some places to 0.01 mm. In the posterior part of the body it is very weakly developed, and in the region of the posterior suckers the fibres are very difficult to detect.

Closely applied to the circular layer of muscles run the second or diagonal fibres (Fig. 10). In the anterior half of the body these, like the transverse fibres, run isolated without forming bundles; and those coming from opposite sides of the body cross each other at an angle of nearly 120°. In the posterior half of the body, this layer is strongly developed in the region of the folds already mentioned, where the fibres run in flat bundles and close to one another. According to Taschenberg,[1] the diagonal fibres are situated innermost in *Tristomum*; but in all the species of ectoparasitic Trematodes I have hitherto examined, viz., in *Microcotyle*, *Axine*, *Octobothrium*,[2] *Dactylogyrus*, and a new genus not yet named, the diagonal fibres are situated between the transverse and longitudinal muscular layers. Lorenz,[3] who includes the transverse and diagonal muscles under one head, also places the "zärteren Fasern" (by which he means the two sets of muscles just mentioned) outward; and an examination of the sections of *Tr. molae*, kindly placed at my disposal by Prof. Ijima, has convinced me of the error of Taschenberg, occasioned perhaps by the circumstance that in *Tristomum* the longitudinal fibres describe a

1) Taschenberg—Beiträge zur Kenntniss ectoporasitischer mariner Trematoden. Halle, 1879. p. 11.

2) In a species of this genus which I have examined, there are in addition isolated longitudinal fibres between the diagonal and circular muscles.

3) Lorenz—Ueber die Organisation der Gattungen Axine u. Microcotyle. Arbeit. a. d. zool.-zoot. Inst. d. Univ. Wien etc. Bd. I. 1878. 3. Heft.

curve in the lateral portions, corresponding to the circular or oval outline of the worm. The same writer did not observe the diagonal fibres in *Onchocotyle appendiculata* and *Pseudocotyle squatinae*[1]; but since they are present in all the species I have examined, they were probably overlooked by him.

Internal to the diagonal muscle, and separated from it by a greater or less amount of connective tissue, run the longitudinal muscular fibres in parallel bundles of greater or less strength. They are more strongly developed on the ventral than on the dorsal side of the body, as is usual in most Trematodes, and cause a slight curve of the body on the ventral side when the worm is killed with hot sublimate. The fibres that constitute the bundles are but loosely united together by connective tissue, and form by no means such compact muscular bundles as we see in some other Trematodes. They appear in cross-section as dots, separated from one another by a greater or less amount of connective tissue between. Some of the fibres of a bundle often diverge from their previous course, and enter into the formation of a neighboring bundle. Most of the longitudinal fibres combine toward the posterior part of the body to form a certain number of strong bundles, which proceed posteriorly, and are inserted one to each sucker on the median chitinous piece of the posterior wall (Fig. 5).

The dorso-ventral muscles (dvm in Figs. 11, 13, 16, 24) are well developed. Each muscle generally breaks up into a few branches dorsally and ventrally before being inserted into the basement membrane. They traverse the brain, vitelline body, and other internal organs. In longitudinal (sagittal) sections of a specimen, in which the vitelline body has not yet well developed, the dorso-ventral muscles are seen to be placed at pretty regular intervals. In

1) Taschenberg—Weitere Beiträge zur Kenntniss ectopar. mar. Trematoden. Halle, 1879.

specimens with fully developed vitelline body, these muscles are obscured to a great extent.

## III. The Organs of Attachment.

The organs of attachment are constituted, posteriorly by the four pairs of suckers already mentioned and a pair of hooks, and anteriorly by a pair of suckers and sticky glands. Each posterior sucker (Figs. 3, 4, 5) may briefly be described as a short-ovate, flat bag with its wide mouth directed ventrally, its walls very thick, and the line of its greatest breadth directed transversely to the long axis of the body; so that we may speak of the anterior (aw), posterior (pw), and lateral walls. The first two walls are very thick, and are directly continuous with each other at the bottom of the bag (Figs. 4, 5). The lateral walls are very thin, and seem to consist of a cuticula-like refractive membrane only. The entire structure is supported by a framework of chitinous rods, which are by no means so numerous or complicated as Nordmann has represented them. They are five in number: a U-shaped median piece (pm), a pair of curved pieces (ppa), (resembling in form certain fishing-hooks), to support the anterior wall, and a pair of similar pieces (ppp), with a large process (pp) at the base, to support the posterior wall. Having thus given a general idea, I shall now proceed to the explanation of the three figures already referred to, by which I hope to make clear the structure of the suckers. Fig. 3 represents the chitinous rods as very commonly seen in a specimen observed under the pressure of a cover-glass, with the mouth of the sucker directed below in the figure and the rods belonging to the anterior wall shaded more deeply. Fig. 4 represents a section made in the direction indicated by the line ab in Fig. 3, whereby it is to be remarked that the median piece has been cut nearer the fundus of the sucker. This section shews the thickness of the anterior and posterior walls, as well as their fibrous structure. The prismatic fibres, of which these walls are composed, are strongly refractive, and are scarcely colored by haematoxylin. They seem, therefore, not to be of the same nature as the muscles of the body; these being well stained by the same coloring fluid. In fact, they seem to be not contractile but elastic fibres. The supporting rods are all of them hollow, with, the inner surface, however, not quite smooth, but with irregular projections, which sometimes unite with those of the opposite side, and form septa-like partitions (Fig. 3). The paired rods are somewhat triangular in section, and are imbedded in their respective walls along the margins. The rods of the posterior wall are articulated at their bases each with another piece (pp), which is imbedded in the substance of the wall, and imparts greater strength to it—a fact well in accordance with the circumstance already mentioned that the main bundle of muscle is attached to this wall. Fig. 5 represents a section made in the direction indicated by xy in Fig. 3, i. e., in an antero-posterior direction. In this section, the direct continuity of the anterior and posterior walls is clearly seen; the U-shaped median piece has been cut but in part, as also the extremities of the paired pieces at the entrance of the sucker. The median piece exhibits, in the posterior wall, a deep cut, where the main bundle of muscle is attached for controlling the varied movements of the sucker. Beside this bundle, weaker ones are attached to the paired pieces. The fibrous substance of the wall is bounded by a cuticula both against the external world and the surrounding mesenchyma. The supporting rods are very easily broken into fragments when the animal is subjected to too much pressure; and this takes place pretty regularly in the manner represented by Nordmann, who, however, describes the fragmentary pieces as "Bügel, zahnförmige Vorsprünge, Rippen, u. s. w." All the posterior suckers are of the same build ; but they vary somewhat in size, the last pair being always smaller than the anterior ones,[1] and the first pair very often smaller than the following two pairs. Measurements on five individuals gave the average breadth of the suckers as 0.093 mm.[2]

Besides the suckers just described, there is, on the dorsal side, a pair of solid chitinous pieces (Fig. 6). Each piece consists of two parts. The basal portion, to which a small bundle of muscle is attached, is straight, and acts as a handle. To this is articulated a hook-like piece, whose end alone sticks out from the surface of the cuticula ; the handle as well as the other part of the hook lying in the integument. The straight handle-like portion and the hook constitute a single piece, and not two pieces as v. Beneden[3] thinks. The total average length of the piece is 0.072 mm.

The anterior suckers are either round or egg-shaped, according to the different states of contraction, and are situated right and left at the entrance of the mouth. Like the posterior suckers, the walls (Fig. 9) are composed of prismatic fibres placed at right angles to the investing membrane, which lines the whole internal cavity, and bounds the wall from the surrounding mesenchyma. In cross-section, the sucker is generally circular in outline. Each is provided with a number of special muscles for the control of its movements in suction. These muscles I have represented in Fig. 12, where there will be seen three bundles coming from the dorsal side, two of which are attached to the anterior border of the sucker, and the remaining one to the

1) This fact must not be taken as proving that the hindermost pair is formed last.

2) A corresponding measurement on the European species of about the same size gave the average result as 0.144 mm. fort he sucker, and 0.084 mm. for the total length of the handle and hook.

3) P. J. v. Beneden—l. c. p. 42.

posterior ventral border. A bundle, which soon divides itself into two smaller bundles, proceeds from the ventral side, and is attached, one of the branches to the same point as the posterior dorsal bundle, the other branch a little more ventrally and anteriorly. Two weaker bundles start, in addition to the above, from the upper and lower lips of the mouth, and are attached to the corresponding borders of the sucker. I have observed some of the fibres of these various bundles directly continued through the substance of the wall, and inserted on the cuticula that lines the cavity of the sucker. By the combined action of these muscles, the worm can exercise a strong suction on the gill of the host, and extract its blood.

Besides these suckers, there is a pair of glands at the entrance of the mouth, just anterior to the suckers, which seem to be peculiar to *Dipl. nipponicum.* They can be seen well in a living specimen under the cover glass, or in preparations of the entire worm, as a round, paired body. One of them is seen in section in Fig. 8, which shews it to be a gland formed by the invagination and local modification of the epidermis. It has generally a reniform cavity, which opens into the mouth by a canal, just anteriorly and close to where the sucker opens into the mouth. The epidermis is continued into the canal for a certain distance, and then changes its character, becoming firmer and refractive like the cuticula. The cavity of the gland is destitute of any distinct epithelium, but is generally filled with a granular mass, which stains very well. This mass is densest near the wall, and gradually becomes thinner towards the centre, where there is generally an empty space. I have often observed the exit canal filled with a deeply stained granular mass, very similar in appearance to the contents of the sticky glands of *Dactylogyrus* and other allied forms, and which is doubtless the sticky secretion of the gland. Next the granular content is a basement membrane. The wall is exceedingly thick and muscular. The muscular fibres are mostly arranged meridionally, i. e. if we suppose the ventral and dorsal pole of the gland to correspond to the two poles of the earth, the muscular fibres are arranged nearly in the plane of the meridians. Fibres also come from the dorsal side of the animal, and enter the wall. Between the muscular fibres, I have sometimes observed nuclei, which are to all intents and purpose exactly similar to those of the general mesenchyma of the body, and probably belong to it.

## IV. The Mesenchyma.

Of the mesenchymatous connective tissue of the Trematodes, Leuckart[1] distinguishes two forms. In the first form, the mesenchyma consists of a "fast homogene helle und feinkörnige Substanz mit zahlreich eingelagerten kleinen Kernen"; in the second form of the mesenchyma, we see "Zellen von mehr oder minder ansehnlicher Grösse, die mit einer meist wasserhellen Masse gefüllt sind" and generally of a polyhedral form, with a fibrous net-work between. Taschenberg[2] regards the mesenchyma "als ein Bindegewebe, welches zu einem Maschenwerke entwickelt ist, in welchem die ursprünglichen Bildungszellen theils noch vorhanden sind, theils aber nur an dem Protoplasma mit darin eingelagerten Kernen sich erkennen lassen." All these forms of the mesenchyma, however different they may seem to be with one another, can, in my opinion, be derived from the differentiation in different directions of a single primitive form. The strong resemblance of the mesenchyma of the Trematodes to the chorda dorsalis of the vertebrates has already been observed by Leuckart; and I believe the former is formed just in the same manner as the latter. But first the mesenchyma of *Diplozoon.*

1) Leuckart—Die Parasiten des Menschen. II. Auflage. I. Bd. 3. Liefg. p. 13 *et seq.*

2) Taschenberg—l. c. p. 13.

In this tissue are imbedded all the organs hereafter to be described, as also some of the organs already described. Owing to the presence of the vitelline body, the mesenchyma in the anterior half of the body is mainly confined to the peripheral portion, but is also present in a scanty quantity between the lobes of the vitelline body and the cells of which they are composed. When one takes it up for study, he finds great perplexity and difficulty in making out the true nature of the elements that compose it, until he compares it with the mesenchyma of other allied species. In *Diplozoon*, it consists of a fibrous substance, in which are seen nuclei each with a distinct membrane of its own. These nuclei always enclose one or more deeply stained nucleoli. The nuclei are of various size and shape. In the anterior portion and generally in the anterior half of the body, they are generally of a comparatively small size (Figs. 7, 8, 9, 25) ; in the posterior half of the body, however, they are generally of a larger size (sometimes having the diameter of about 0.01 mm.) and have a circular or oval outline (Fig. 13). In the vicinity of the internal organs, where the connective tissue is generally more or less compressed, the nuclei are smaller and often fusiform in shape. Around the pharynx, the fibres form a fine close net-work (Fig. 11).

Beside these elements, we see here and there, scattered apparently without any regularity in the parenchyma, large vesicular bodies of a circular or oval outline (Fig. 13), with a large conspicuous nucleus in the centre surrounded by a mass of granular protoplasm, which on close inspection betrays a fibrous structure, and which gradually thins out peripherally, and leaves an empty space between it and the wall. These vesicular bodies are sometimes drawn out towards one end, and are very abundant in the posterior half of the body, posterior to the testis. In the region situated between the ovary and the testis, the mesenchyma consists of distinct cells with a granular, generally well-stained protoplasm, of a polyhedral form, and leaving irregular intercellular spaces between (Fig. 14).

In *Axine*, the mesenchyma is distinctly seen to consist of large, vesicular cells, each with a nucleus generally in the centre, but sometimes attached to the wall, and filled with a hyaline fluid containing numerous almost uncolored granules. The nucleus as in *Diplozoon*, has a distinct membrane, and encloses a deeply stained nucleolus, but is considerably smaller. Beside these cells, there are, as Lorenz[1] has already observed, in the neighbourhood of the vagina, cells whose contents take up the staining fluid very eagerly and appear like ganglion cells. In *Microcotyle*, the mesenchyma presents somewhat different aspects in different parts of the body— a statement that holds good to a greater or less extent in all other allied forms. Around and outside the vitelline body, the mesenchyma presents an appearance very similar to that of *Diplozoon*. Nearer the median line, it consists of large cells with the nuclei in the centre, from which protoplasmic fibres radiate to the wall, whose cavity is filled with a clear fluid without any granule. Along the median line, finally, the mesenchyma consists of cells with a granular somewhat fibrous protoplasm which deeply stains with haematoxylin.[2] Here in *Microcotyle*, I believe, are manifested the transitional steps through which the mesenchymatous connective tissue such as that of *Diplozoon* has been differentiated from the primitive parenchyma cells. These primitive cells are, I believe, very nearly represented by the cells of the median portion of *Microcotyle*. The next step onward toward the differentiation of connective tissue is, according to my view, represented by such a form of mesenchyma as that of *Axine*, or that portion of the same in *Microcotyle* situated just inside the vitelline body— composed of cells of a vesicular appearance

1) Lorenz—l. c. p. 7.

2) In appearance, these cells are very similar to the yolk-cells of *Diplozoon* during the winter season. *Vide* Fig. 20, Pl. XXII.

and filled with a hyaline fluid. A step further onward in the same direction would result in the formation of abundant fibres, and the boundaries of the original cells would be partly absorbed and entirely obliterated ; so that we should then have a ground-mass of irregular fibrous substance, with nuclei scattered therein— in fact just such a form of mesenchyma as we really see in most parts of the body of *Diplozoon.* The large, round, vesicular bodies above mentioned (Fig. 13) are in fact the remnant cells of the original parenchyma, and the portion, already referred to, situated between the ovary and the testis, seems to have undergone but little transformation, and to have preserved the original cellular structure. According to the view here stated, the so-called pseudocoel of the Trematodes would be *not* spaces formed by the departing of the cells from one another leaving intercellular spaces between them, but spaces which were before truly *intra*-cellular. I do not, indeed, entirely deny the presence of truly *inter*-cellular spaces, but these are, I believe, comparatively insignificant.

Similarly, of the two typical forms of Leuckart, the first results apparently by a simple obliteration of the boundaries of the original cells. The second form can be derived by a process similar to that which we have seen to have taken place in *Microcotyle*, in which some of the cells (the larger vesicular ones) have maintained their cellular nature more completely, while others have been more or less completely transformed into connective tissue, and pressed in, forming the "Maschengewebe," between the former ; as already proved embryologically by Schwarze.[1] The two forms above mentioned, are connected by numerous intermediate forms, and an actual transition between them has been observed in some species.[2]

1) Schwarze—l. c. p. 59.

2) Looss —Beiträge zur Kenntniss der Trematoden. Ztschr. f. w. Zool. Bd. XLI. 1885. p. 432.

Beside the various elements hitherto described, there are, in the neighborhood of the brain and pharynx, large cells of a roundish or polygonal outline, easily distinguishable from the surrounding elements of the parenchyma (Fig. 25). They are of a gigantic size, and in some sections they seemed as if they were drawn out into fibres in more than one direction. They have conspicuous vesicular nuclei enclosing each a deeply stained nucleolus, which again generally encloses a vacuole. They are entirely destitute of cell-walls, and have a finely granular protoplasm. Their very appearance suggests their nervous nature. But more than that, careful examinations have convinced me that these large cells are very constant in their position and number. They are found, namely, laterally and behind the pharynx, and can be seen in living specimens under the cover-glass, especially well after the water has evaporated to a certain extent. As will be seen from the figure, they are situated symmetrically, right and left, on both sides of the pharynx. Besides the four cells on each side and a median ventral one, drawn in the figure, I have counted another pair and a median unpaired one more posteriorly. There are also similar cells, which are scattered apparently without symmetry, around the brain, but always outside it in the mesenchyma. Two of these are shewn in Fig. 17.

Considering the form and appearance of these cells, the constancy and symmetry of their position and number (at least in the more anterior ones), together with the circumstance that there are no nervous cells *in* the brain or nerves, I am strongly inclined to attribute nervous functions to these gigantic cells ; but I have not been able to trace any direct connection with the nervous system. I have tried methyl-violet and cochineal stain. By the latter, they are but slightly colored, and neither of these stains affords any better clue into their exact nature. They seem to be different from the remnant cells of the parenchyma already described (Fig. 13); but I must leave the exact nature and function of these cells undetermined.

## V. The Digestive System.

The digestive system consists of the mouth (Fig. 2, mo), the prepharynx (pph), the pharynx (ph), the oesophagus (oe), and the intestine (int).

The mouth is a funnel-shaped opening situated on the ventral side of the anterior extremity of the body, at the entrance of which are placed the glands and suckers already described. Its cavity is lined by the continuation of the cuticula of the general surface of the body. The fundus of the funnel leads directly into an expanded cavity, the prepharynx, into which the anterior half of the pharynx protrudes. This latter is an ellipsoidal body which has a narrow tubular cavity passing through the centre, and whose major axis is directed antero-posteriorly. In cross-section (Fig. 11) it is circular. The internal tubular cavity is lined by a comparatively thick structureless membrane. The thick wall is composed of muscular fibres arranged in regular groups, and of connective tissue, in which nuclei, very similar to those of the general mesenchyma, are to be observed. Most internally, and separated from the structureless membrane lining the internal cavity by a sort of basement membrane, is a thin layer of circular fibres (mci). Most externally, and directly internal to the cuticula-like membrane that envelopes the whole pharynx and separates it from the surrounding mesenchyma, is another layer of circular fibres, about double as thick as the first. Besides these, there are radial fibres extending between the internal basement membrane and the external cuticula of the pharyngeal wall. These radial fibres are weakly developed, and do not run in bundles, as they have been observed to do in some other Trematodes. Between these fibres is found a mass of connective tissue with conspicuous nuclei. These nuclei are doubtless the remnants of the cells that produced the muscular fibres and the connective tissue of the pharynx. Strong dorso-ventral muscular bundles (Fig. 11, dvm) are closely applied to the wall of the pharynx, and no doubt assist in its action. The total thickness of the pharyngeal wall, the internal membrane inclusive, is about 0.02 mm.

The cavity of the pharynx leads directly into the oesophagus, a simple, slender, tubular portion, which is directly continued into the median trunk of the intestine. This median trunk sends out in the anterior half of the body, right and left, lateral branches, which ramify dichotomously once or twice. Some of these lateral branches are distinctly paired, but I have also observed others which are as distinctly unpaired. Posterior to the place of crossing of the two individuals the lateral branches are absent. Here the median trunk divides into two, one of which retains nearly the median position, while the other proceeds more laterally towards the ovary. Posterior to the testis these two branches unite, and thenceforth the intestine proceeds towards the suckers as a simple unbranched tube, and ends between and a little anterior to the first pair of suckers, where it generally presents a rounded enlargement. "A l'endroit ou les deux corps s'unissent, les coecums digestifs semblent atrophiés, mais en dessous de l'appareil générateur, dans le bout postérieur du corps, chaque tube présente de nouveau ses ramifications régulières et complètement séparées, comme dans la partie antérieure," says v. Beneden,[1] and I can confirm his observation with my own on the European species ; but in *nipponicum* I have found this part of the intestine always simple. The wall of the intestine is destitute of an epithelium

1) P. J. v. Beneden—l, c. p. 40.

such as we are wont to see in the Distomes. In its stead, we find large cells (Figs. 14, 16, 19, dc) separated from one another by a considerable interval, and filled with dark-brown or sometimes even black granules. I have not observed any wall or nucleus in these cells, although Zeller[1] points to the presence of the latter in *Polystomum*, and I could distinctly observe it in *Octobothrium*. These black pigment-containing cells I hold, in agreement with Taschenberg,[2] to be digestive cells, and the pigment-granules to be food-particles taken in from the cavity of the intestine. Digestion, therefore, takes place in the allied forms intracellulary, as in the Turbellarians. The intervals between these cells are usually destitute of any distinct membrane in the anterior half of the body, so that here the digestive system consists of mere hollows in the mesenchyma ; but in the posterior part, where the intestine is simple, I could usually distinguish a more or less distinct membrane of compact connective tissue.

## VI. The Excretory System.

The excretory system of the Plathelminthes has been minutely examined by Fraipont,[3] Lang,[4] Pintner[5] R. Wright and Macallum,[6] and some others. By these investigations two points seem to have been firmly established : 1) That the excretory system

1) Zeller—Untersuch. ü. d. Entwick. u. d. Bau. d Polystomum integerrimum. Ztschr. f. w. Zool. Bd. XXII. 1872. p. 19.

2) Taschenberg—Weitere Beiträge. p. 11.

3) Fraipont—Recherches sur l'appareil excréteur des Trématodes. Archiv. d. Biologie. T. I. I have not been able to gain access to this work, and am indebted for its account to J. V Carus's "Zoologischer Jahresbericht" (1880. 1. p. 277) and to Looss (l. c.).

4) Lang—Der Bau von Gunda segmentata u. d. Verwandtschaft etc. Mittheil. a. d. zool. Station z. Neapel. Bd. III. 1882. p. 187.

5) Pintner—Untersuch. ü. d. Bau. d. Bandwurmkörpers, mit bes. Berücksichtigung etc. Arbeit. a. d. zool.-zoot. Inst. d. Univ. Wien, etc. Bd. III. 1880. 2. Heft.

6) R. Wright and Macallum—l. c. p. 20.

of this class consists of vessels with a distinct wall, 2) that these vessels are of two kinds, the larger ones serving mainly for leading out the contained fluid, and the capillaries which end in funnel-shaped little bodies shewing the so-called "Wimperflamme" in the interior, and which are the most important part of the system.

In *Dipl. nipponicum*, as in *Dipl. paradoxum*, two main canals can always be distinguished on each side of the body, one of which is larger than the other and opens to the exterior by means of a circular opening on the dorsal side[1], close to the lateral margin, a short distance posterior to the pharynx (Fig. 2, eo). Immediately at the entrance of the opening, the vessel presents an enlargement (the so-called "Sammelrohr"), then proceeds anteriorly to about the level of the pharynx, where it bends backward and proceeds posteriorly, winding more or less on the way, and giving off but a few branches. On reaching the posterior suckers, it bends inward to them and reaches nearly the posterior margin of the body. Here it turns on itself and proceeds anteriorly, following closely its former course, but this time liberally sending out branches which anastomose with one another and with those from the opposite side of the body. Anteriorly this main vessel reaches the upper lip of the mouth, where it divides itself into numerous branches, having also become smaller during its course. These two main vessels follow closely in their course that of the ventral nerves, on whose dorsal side they are situated except where they make windings towards one side or the other. I have sometimes observed a direct connecting vessel between the two main ones. Within these vessels as well as the branches that proceed from them are seen, in a living specimen, active vibratory movements, which generally come to view only after the animal has been left for

1) Cf. Braun—Ueber die Lage d. Excretionspori bei d. ectopar. Trematoden. Zool. Anz. Jahrg. XII. 1889. p. 620.

some time under the cover-glass, and which are executed in such a way as to drive the contained fluid towards the excretory pores. These movements are probably due to the presence of vibratile flaps in the wall, but I have not been able to observe them in sections. The wall is seen, in section, to be formed by a compact refractive membrane with double contour, which does not stain with haematoxylin (Fig. 16, as). Evidences have been advanced by Lang[1] and Ijima[2] that the excretory vessels of the Turbellarians are "nichts Anderes als durchbohrte Zellen." In the Cestodes, Pintner[3] has observed a well-developed epithelium on the wall of the main vessels, "das zweifelsohne als Matrix ihrer glashellen, homogenen Membran aufzufassen ist." According to Schwarze[4], the central excretory vessel of the Distomes is at first a solid string of cells, which afterward acquires a lumen. He also supposes that the finer branches originate in the same way, and that the structureless condition of their walls in the adult worm may be explained by supposing "dass nach der Resorption des Inhaltes der primären Zellen keine äussere, muskulöse Zellenlage gebildet wird, *sondern die Wandung sich allein aus der äusseren Zellmembranen der ursprünglichen Anlage zusammensetzt.*" Whether the walls of the excretory vessels of *Diplozoon* is to be regarded as similar to those of the Turbellarians, with the difference that the protoplasmic remnants of the original cells have been transformed into a structureless membrane, or whether they had been produced by a distinct epithelium which afterwards underwent degeneration and finally disappeared, or whether they were formed by such a process as Schwarze[5] supposes

1) Lang—l. c. p. 212.

2) Ijima—Untersuch. ü. d. Bau u. d. Entwickl. d. Süsswasser-Dendrocoelen. Ztschr. f. w. Zool. Bd. XL. 1884. p. 397.

3) Pintner—l. c. p. 21.

4) Schwarze—l. c. p. 58. The italics are mine.

5) In the extreme case, viz. where the cells are arranged in a single row, the view of Schwarze reduces itself to that of Lang and Ijima.

to have taken place in the smaller excretory vessels of the Distomes, I must leave entirely undecided, with the single remark, however, that in *Diplozoon* I have observed no trace of nuclei in the wall.

The capillaries, furnished like the larger vessels with a distinct wall of compact refractive membrane, proceed from the smaller branches of the main vessels, and continue throughout their whole course without undergoing any perceptible diminution of their calibre. They are especially abundant in the layer of the mesenchyma just under the muscular wall of the body. They do not, like the branches of the larger vessels, anastomose with one another, and no vibratory movement is to be observed within. They branch freely, and each of the branches ends with a minute funnel-shaped enlargement (Fig. 15), within which is to be seen an active vibratile flap, the so-called "Wimperflamme." Various structures have been described in connection with these funnels, but, although I directed my utmost attention to the point and applied the best lenses at my disposal (Seibert apochr. syst., 4mm × 8), I could not observe any of them. The majority of the writers who have specially investigated this subject seem to agree in excluding any direct communication between the cavities of the funnels and those of the surrounding mesenchyma. In this respect, however, Fraipont makes an exception. He observed "fenètre ovale" in the wall of the funnel, by which its cavity was put in direct connection with the surrounding pseudocoel. I had at first supposed the end of the funnel completely closed; but on repeated observations with the apochromatic system of Seibert, it seemed to me very probably open, and to communicate with the cavities of the mesenchyma. I have not observed any of those peculiar cells described by preceeding writers.[1]

[1] On reading Wright and Macallum's description, the question naturally arises if the writers have not mistaken the ciliated portions of the capillaries, such as have been described

## VII. The Nervous System.

With the excellent investigation of Lang[1] on the nervous system of the Trematodes before me, I directed my special attention to this system, and can confirm his statement in its general aspect, though it seems to me to require modification when the writer extends it to the Trematodes in general. Let us begin with the brain.

As to its position, Lang says, "Ich glaube überhaupt, dass bei allen Trematoden das Gehirn diese Lage hat, dass es nämlich bogenförmig über den vorderen Theil des Pharynx verläuft und ich zweifle, ob sich die abweichenden Angaben bei erneuter, genauer Untersuchung bestätigen würden." Leuckart[2] is inclined to explain those cases where the brain has been observed behind the pharynx "durch eine Lagenveränderung" of the latter, "die um so leichter eintreten kann, als das Nervenband nirgends ringförmig geschlossen ist, obwohl das für einzelnen Arten behauptet wurde." I find, however, after careful and repeated observations, with these statements full in view, that in *Diplozoon* and also in *Axine*, *Microcotyle*, and *Octobothrium*, the brain is a band-shaped nervous body arching over the oesophagus on the dorsal side and *behind* the pharynx. In a fresh specimen, it is seen to be composed of very thin fibres; but sections shew that in addition to these fibres the brain contains a finely granular substance doubtless identical with the "Punktsubstanz" of the Turbellarians (Fig. 17). The fibres in the brain are seen to run mainly in two groups, one on

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by Looss, for the funnels, and overlooked these latter. I also belive that they go too far when they endeavor to attribute excretory nature to the large cells observed by Looss and others in the pharynx of many Trematodes.

1) Lang—Untersuch. z. vergleich. Anatomie u. Histologie d. Nervensystems d. Plathelminthen. Mitth. a. d. zool. Station z. Neapel. Bd. II. 1881. p. 28.

2) Leuckart—l.c. p. 22.

its dorsal side, the other more on the ventral side, close to the dorsal side of the oesophagus. These I have marked in the figure with a lighter shade. They unite at the two ends of the brain where the nerves take their rise. The brain is traversed by numerous dorso-ventral bundles as already mentioned.

From the brain are given off nerves both anteriorly and posteriorly. One pair (Fig. 2. nai) proceeds anteriorly near the median line embracing the pharynx, near the anterior part of which it is lost in the mesenchyma. A second pair (nae) proceeds more laterally, and can be followed as far as the suckers, externally to which it proceeds and there withdraws itself from view. These internal and external anterior nerves are connected with each other by a commissure at a little distance from their origin in the brain.

Two pairs of nerves also proceed posteriorly, one of which may be called the ventral pair and is by far the stronger pair. The other pair (nvl) may be called the ventro-lateral nerves and proceeds posteriorly just at the angle between the ventral and lateral borders of the body, and can be followed as far as where the two individuals cross each other. The ventral nerves (nv) take their rise in the brain at its postero-lateral corner, and can be followed to near the posterior border of the body. They become, however, more and more indistinct as they proceed posteriorly, and finally become invisible at about the level of the hindermost pair of suckers. They closely follow in their course the main excretory vessels, on whose ventral side they are situated at a little distance from the muscular layers. At the place where the two individuals cross each other and where the ventro-lateral nerves withdraw themselves from view, the ventral nerves take a more lateral position, and this position they keep throughout the remainder of their course. The ventral nerves are connected with each other and with the ventro-lateral nerves by a number of commissures occurring nearly at regular intervals; and in such a way that each commissure between the ventral nerves lies in a line with that between them and the ventro-lateral nerves. The ventro-lateral nerves, again, sends out branches towards the lateral margin of the body, just at those points where they receive the commissures from the ventral nerves; so that all the nerves form a regular rectangular net-work, and divide the whole ventral surface of the body into a number of distinct areas. At the points where the commissures cross the main nerves, the course of the fibres is interesting. From any main nerve, namely, which we may be considering, fibres are given off on both sides to the neighboring nerves. Beside these, there are also fibres coming from the latter and proceeding directly past the main nerve without mingling with its fibres, so that the four main nerves are probably put in direct connection with one another. I have counted as many as thirteen commissures in the anterior half of the body, in addition to the pair of commissures between the anterior nerves. In the posterior half the commissures seem to be less numerous. I have been able to count only a few; but this is perhaps due to the presence of the strong folds already mentioned and the special development of the diagonal muscle in this region, which greatly increases the difficulty of following the course of the nerves. I have not been able to make out the plexus which the nerves probably form on the dorsal side.

As to their histological character, the nerves present typical "Balkenstränge" (Fig. 16). In some of the meshes are to be seen sections of nervous fibrils as exceedingly minute dots, which are visible only in the most favorable cases. Pintner[1] maintains that the "Bälkchen selbst" which form the mesh-work are the sections of the fibrils which are probably arranged "reihenweise, nebeneinand-

1) Pintner—l.c. p. 71.

erstehend." Poirier[1] describes the nervous fibres of *D stonum clavatum* as filling up the entire cavity of the meshes. But an examination of the nerves in a fresh state shews very distinctly the exceedingly fine fibrils. They do not seem to be so regularly arranged as Pintner supposes, and are not at all large enough to fill up the entire cavities of the meshes. Without doubting the correctness of Poirier's observation, I am convinced that in *Diplozoon* the nerves consist of a frame-work of connective tissue, in the meshes of which run the true nervous fibrils. I have not observed any of the nervous cells described by Lang and others in the nerves. This set me to a careful search after ganglion cells, as these were not also to be found in the brain, where in other species they make such a conspicuous figure especially in the peripheral portion. But I have not been able to find out any to which I could decidedly point as nervous cells (*Vide supra* p. 171).

## VII. The Reproductive System.

We now come to the consideration of the most complicated system, the reproductive organs. Of these the female portion consists of the vitelline body, the ovary, the oviduct, and the uterus, with a "connecting canal" the nature of which is not at all clearly known. The male portion consists of the testis with a single vas deferens. I shall begin with the latter.

*The Male Organs*—The testis is a nearly globular or ovoid body situated about midway between the point of crossing of the two individuals and the posterior margin of the body, and is composed of many lobes. Each lobe is separated from its neighbour and from

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[1] Poirier—Contribution à l'histoire naturelle des Trématodes. Arch. d. zool. expérimentale 2e. Série. T. III. 1885. p. 603.

the surrounding mesenchyma by a layer of dense connective tissue (Fig. 2, t & Fig. 18). During the winter season, it is a solid mass of vesicular cells that have assumed a polyhedral form by their mutual pressure. Each cell encloses a conspicuous round nucleus, which seems to be provided with a wall of its own, and in which numerous chromatin particles are to be observed. The cytoplasma is a hyaline fluid which scarcely takes up any color. The nuclei are of various sizes in the same lobe, some being very small, leaving abundant space for the cytoplasma, while others are of such a size as nearly to fill up the entire cavity of the cell.

From the anterior end of the testis proceeds a single vas deferens, which passes anteriorly in a straight course dorsal to the oviduct and ventral to the yolk-duct. During the first part of its course, it lies ventrally to the uterus ; but at about the level of the anterior end of the ovary, it turns dorsal to it and *opens into the vitelline duct of the other individual* a little more anteriorly than the anterior end of the ovary. Zeller[1] represents the vas deferens of one individual as standing in direct connection with the "Laurer's canal" of the other. But my observations contradict this view entirely. I have traced the course of the vas deferens in more than one series of the sagittal sections of the worm. One of these series is reproduced without interruption on Pl. XXIII. The opening of the vas deferens of one individual into the yolk-duct of the other is seen in Figs. XI. & XIII. By the same series of sections, the opening of the connecting canal of the oviduct into the intestine is distinctly seen (Figs. V & XIX). The vas deferens is destitute of any distinct wall of its own. It seems to be merely a continuous tube-like cavity in the general mesenchyma, and to collapse entirely during the winter season.

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1) Zeller—Ueber den Geschlechtsapparat des *Dipl. paradoxum*. Ztschr. f. w. Zool. Bd. XLVI. 1888. p. 233.

*The Female Organs*—The ovary (Fig. 2, ov) is a long conico-cylindrical body which is doubled on itself by its middle portion, so that the two ends come close to each other, and placed on the dorsal side of the body just anterior to the testis, to which its smaller end is closely applied ; the anterior end where it is doubled on itself reaching as far as where the dorsal papilla formerly was. From its larger end, where ripe ova are found, proceeds the oviduct. As we approach the other end, the ova become smaller and smaller until finally we see a mere assemblage of round nuclei imbedded in a common mass of protoplasm. The whole ovary lies in a mere cavity of the mesenchyma without any distinct wall of its own. A section through the larger end (Fig. 21) shews the ovary to be a solid body consisting of large ova which are either polygonal or wedge-shaped according to the direction of the section. Each ovum is destitute of any membrane, and consists of a mass of homogeneous deeply stained protoplasm, in which lies a large vesicular nucleus provided with a distinct wall and containing a hyaline fluid in which float numerous deeply stained dots, the chromatin particles. Each nucleus again encloses a large deeply stained nucleolus in which are again to be observed one large or a few smaller vacuoles. Zeller[1] mentions and figures in the ovum of *Polystomum* and *Diplozoon* a thick, elastic "Hülle" ; but I have no doubt that the ovarian ova of *Diplozoon* are destitute of any membrane. This is also the case in *Axine*, *Microcotyle*, *Octobothrium*, *Dactylogyrus*, in fact in all the species of ectoparasitic Trematodes I have hitherto examined. Willemoes-Suhm[2] mentions no "Dotterhaut" in *Polystomum ocellatum ;* Taschenberg[3] asserts

1) Zeller—Ztschr. f. w. Z. Bd. XXII. p. 5 & 169 foot-note; Bd. XLVI. p. 235. Is not the elastic membrane the result of fertilisation?

2) Willemoes-Suhm—Zur Naturgesch. d. Polyst. integerrimum u. Polyst. ocellatum. Ztschr. f. w. Z. Bd. XXII. 1872. p. 33.

3) Taschenberg—Beiträge. p. 36.

the absence of any membrane in the ovum of *Tristomum;* so also Wierzejski[1] in that of *Calicotyle Kroyeri;* and I believe the same is true of the ova of all ectoparasitic Trematodes. As we proceed nearer the smaller end of the ovary, the ova and their nuclei become smaller and smaller, the vacuoles within the nucleolus disappear and finally the nucleolus itself, until we see only spherical nuclei crowded together and surrounded by a common mass of uniform protoplasm. Fig. 22 shews a section through this part.

The oviduct proceeds from the larger end of the ovary and takes its course posteriorly and to the right, ventral to the vas deferens and the testis. At a short distance from its origin, it receives a canal (Fig. 2, cc) which proceeds anteriorly and, after making a slight winding or two, opens into the intestine (Figs. V & XIX). This is the "Laurer's canal" of Zeller which he represents as standing in direct connection with the vas deferens of the other individual. In *Polystomum integerrimum*[2] he asserts a direct connection between the ovary and the testis; and in proof of this he alleges his observation of the ova passing through the oviduct and Laurer's canal and entering the cavity of the testis. But it has been pointed out by Ijima[3] that the canal in question distinctly opens into the intestine, and that a similar canal is present in many other species of the group; and I can confidently state from my own study that the "dritte Dottergang" of Lorenz in *Axine* and *Microcotyle* distinctly opens into the intestine. A similar connecting canal is also present in a species of *Octobothrium* which I have examined.[4] The fact cited by Zeller can be explained

1) Wierzejski—l. c. p. 558.

2) Zeller—Weiterer Beitrag z. Kenntniss d. Polystomeen. Ztschr. f. w. Z. Bd. XXVII. 1876. p. 245.

3) Ijima—Über den Zusammenhang d. Eileiters mit d. Verdauungscanal bei gewissen Polystomeen. Zool. Anz. Jahrg. VII. 1884. p. 635.

4) Voeltzkorw (Arb. a. d. zool.-zoot. Inst. in Würzburg. Bd. VIII. 1888. p. 267) describes an evidently homologous canal in Aspidogaster conchicola. According to him it ends blindly near the dorsal surface of the worm. He calls it Receptaculum vitelli.

if we consider that the intestine is destitute of any distinct wall, and that when the testis is nearly empty there is almost nothing that would prevent the entrance of the ova into the cavity of the testis by way of the intestine. I therefore believe, notwithstanding his positive statement to the contrary, that the canal in question opens also in *Polystomum* into the intestine at the point where he represents it as arising from the testis. In *Dactylogyrus* a similar canal opens externally on the dorsal side, at a short distance from the right lateral margin of the body. In *Dipl. paradoxum* this canal is very long and undergoes numerous convolutions, but in *nipponicum* it is shorter and nearly straight, and the internal surface is clothed with cilia. Its nature and function, if it has any, I hope to be able to treat of later. At a little distance from the point where it receives this canal, the oviduct receives also the yolk-duct (yd). After this it continues its former course, and then, making a sudden turn anteriorly, opens into the uterus.

The uterus, under which I include both the "Ootyp" and the "Eiergang" of the German writers, is a cylindrical tube with a distinct wall which is thickly beset for the greater part of its length with long cilia on its internal surface. It shews an ovoidal enlargement at its origin, the "Ootyp," then diminishing in diameter proceeds anteriorly, following the same course as the vas deferens, and opens externally by a small aperture on the ventral side just at the angle formed by the ventral side of one individual with the dorsal side of the other, at the top of a conical elevation which is sometimes very small, sometimes larger and very conspicuous. Just before opening, it presents a second enlargement in which a single egg is usually found during the period of reproductive activity. "Il y a à l'origine de ce conduit (i. e., of the uterus) une sorte de pylore," says v. Beneden.[1]

1) v. Beneden—l. c. p. 43.

This is caused by the opening at this point of numerous flask-shaped unicellular glands (shg), the shell-glands. The wall of the uterus proper (Ootyp, Fig. 24) is lined by a distinct epithelium, whose cells contain each a round nucleus projecting into the internal cavity. The protoplasm is granular and no cell-boundaries are to be seen. The epithelium sits on a distinct basement membrane and is destitute of cilia. The remainder of the uterus (Eiergang) is provided with a similar wall (Fig. 23), with the nuclei, however, more separated from one another. Here, as already stated, the wall is beset with long cilia.

The vitelline body is an extensive lobed body (Fig. 2, vb) situated exclusively in the anterior half of the body, all around the intestine both on its dorsal and ventral sides. In specimens in which reproduction is going on, each lobe is seen, when fresh, to contain a dark granular mass. Sections (Fig. 19) shew that each lobe consists of a number of cells containing numerous yellowish granules, each with a nucleus and a nucleolus in the centre, and a thin cell-wall. These are the ripe yolk-cells, and when freed take up a globular form. In the peripheral portion are seen smaller cells with a deeply stained protoplasm, a nucleus and a nucleolus. The protoplasm is homogeneous, finely or coarsely granular according to their different stages of development. They are the young yolk-cells ; and there are also to be observed cells intermediate between these two kinds—cells one half of whose content has already been changed into yellowish yolk-granules while the other half still consists of granular protoplasm. During the winter months, the yolk-cells present a quite different appearance (Fig. 20). They are then scarcely to be distinguished from the cells of the mesenchyma of certain species of *Microcotyle*. They are then of a polygonal form, with a distinct cell-wall, a round nucleus and nucleolus, and a granular protoplasm which stains very well. In this granular protoplasm there are fibrous structures radiating from the central nucleus to the cell-wall and more or less forming a net-work. The steps by which these cells are changed into ripe yolk-cells and the origin of the deeply stained young yolk-cells I must leave at present unexplained.

As will be immediately seen from the above investigation, the union of *Diplozoon* is, as Zeller maintains, a permanent copulation. But the relation in which he has represented the parts of the two individuals to stand to each other requires correction. We have seen that the vas deferens of one individual opens into the yolk-duct of the other. This is well in accordance with the probable mode of copulation in some allied forms. In *Microcotyle*, which seems to be very closely allied to *Diplozoon*, there is a dorsal vagina which leads into a canal opening into the yolk-duct. In this canal I have often observed spermatozoa, and as during the period of reproductive activity yolk-cells are constantly going down the yolk-duct and push down before them anything that might come up from below, it is very probable that these spermatozoa had found their way here from the dorsal vagina. Hence the supposition is very natural that in copulation the penis of one worm is directly applied to the dorsal vaginal opening of the other. Now if this very probable supposition be true, and if we further imagine such a relation to persist permanently, we should have just the case that we actually see in *Diplozoon*, with the only difference that the copulation is not cross-wise. Whether in *Microcotyle* also, as in *Polystomum*, the copulation is normally cross-wise and mutual is well worthy of our attentive observation, since if this be the case, the copulation of *Diplozoon* would be nothing more or less than *the regular mode of copulation in allied forms made permanent.*

In conclusion I wish to express my best thanks to Prof. K. Mitsukuri and Prof. C. G. Knott for kindly looking through my paper and making suggestions.

*Tokyo, October 1890.*

## Explanation of Figures.

Abbreviations common to all the figures.

*as*.........ascending stem of the excretory vessel (according to the direction in which the contained fluid moves).

*br*.........brain.

*cc*.........connecting canal between the oviduct and the intestine.

*dvm*.......dorso-ventral muscle.

*ds*.........descending stem of the excretory vessel.

*dc*.........digestive cell.

*eo*..........excretory opening.

*int*.........intestine.

*mce*........external circular muscle } of the pharyngeal wall.

*mci* ........internal „ „ } of the pharyngeal wall.

*mo*.........mouth.

*nae*........external anterior nerve.

*nai*........internal anterior nerve.

*nv*.........ventral nerve.

*nvl*.........ventro-lateral nerve.

*ov*.........ovary.

*ovd*.........oviduct.

*oe*.........oesophagus.

*ph*.........pharynx.

*pph*........prepharynx.

| | | |
|---|---|---|
| *pm*.........median piece | | |
| *ppa*........paired anterior piece | of the posterior suckers. | |
| *ppp*........ „ posterior „ | | |
| *pp*.........process of the posterior piece | | |

*sa*.........anterior sucker.
*sp*...........posterior sucker.
*sg*.........sticky gland.
*shg*.........shell gland.
*t*..........testis.
*ut*.........uterus.
*vd*.........vas deferens.
*vb*.........vitelline body.
*yd*.........yolk-duct.

All the figures, if not otherwise stated, were drawn with cam. luc., Zeiss E×2.

## Pl. XXI.

*Fig.* 1.—Dipl. nipponicum killed with boiling sublimate; free-hand, surface view, × about 14. The black dots represent the digestive cells seen through the tissues.

*Fig.* 2.—The same, free-hand, from a specimen killed under the cover-glass, shewing the internal organs, half-diagramatic. The right anterior half presents the ventral, and the corresponding posterior half the dorsal aspect; and *vice versa* with the other individual. The nerves are colored yellow; the excretory vessels indigo-blue.

*Fig.* 3.—Chitinous frame-work of the posterior sucker as seen in a specimen under the cover-glass.

*Fig.* 4.—Section of the posterior sucker in the direction indicated by ab in Fig. 3.

By inadvertence of the printers, the nucleoli in Figs. 8, 9, 11, 13, 16, 17, 18, 19, 20, 23, and 24 are represented either as lying outside the nuclei or quite eccentrically in them, whereas they ought to occupy more central positions. Their true positions are indicated in most of the figures by weakly shaded dots.

*Fig.* 5.—Section of the same in the direction indicated by xy in Fig. 3.

*Fig.* 6.—Hooks between the posterior suckers.

## Pl. XXII.

*Fig.* 7.—A part of a cross-section of the worm passing through one of the conical elevations of the epidermis. It also passes through one of the transverse folds into which the surface of the body is thrown when the animal contracts; hence the longitudinal muscles are separated from the circular by a rather thick layer of connective tissue.

*Fig.* 8.—Section of the sticky gland, from a cross-section of the worm.

*Fig.* 9.—Section of the anterior sucker, from a cross-section of the worm.

*Fig.* 10.—To shew the direction of the diagonal muscular fibres, from a horizontal section of the worm.

*Fig.* 11.—Cross-section of the pharynx.

*Fig.* 12.—Diagram shewing the muscles accessory to the pharynx.

*Fig.* 13.—A part of a cross-section of the worm, from the posterior half of the body, a little posterior to the testis; to shew the character of the mesenchyma.

*Fig.* 14.—The portion of the mesenchyma situated between the ovary and the testis, from a sagittal section of the worm.

*Fig.* 15.—Excretory funnel, Seibert apochr. sys. 4 mm. × 8.

*Fig.* 16.—Cross-section of the ventral nerve.

*Fig.* 17.—The brain, from a cross-section of the worm.

*Fig.* 18.—The testis, from a longitudinal section of the worm.

*Fig.* 19.—The vitelline body, from a longitudinal section of a worm collected in September.

*Fig.* 20.—The same, from a cross-section of a worm collected in May; the yolk-cells not ripe.

*Fig.* 21.—Section of the ovary near its larger end.

*Fig.* 22.—The same near its smaller end.

*Fig.* 23.—Longitudinal section of the uterus (Eiergang).

*Fig.* 24.—Cross-section of the uterus proper (Ootyp).

*Fig.* 25.—A cross-section of the worm through the region of the pharynx ; to shew the peculiar gigantic cells. Zeiss D × 2.

## Pl. XXIII.

An uninterrupted series of sagittal sections of the worm. To the respective abbreviations is subjoined the letter *r* or *l* according as the parts belong to one or the other individual. Zeiss B × 2.

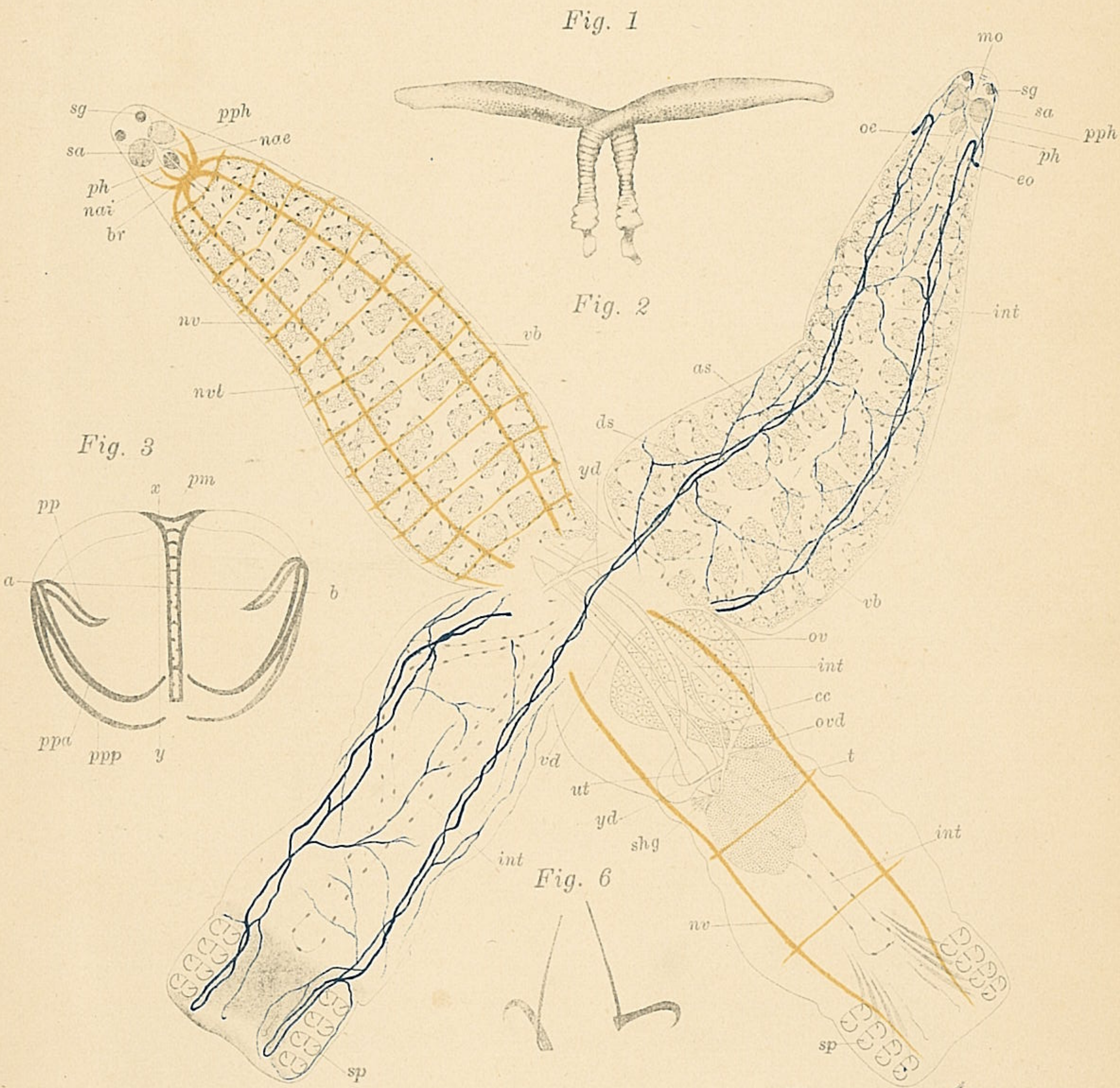



Fig. 4

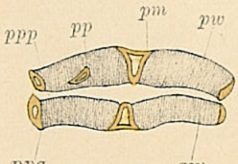
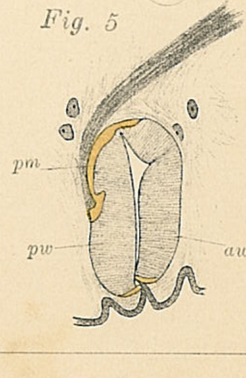



Fig. 5

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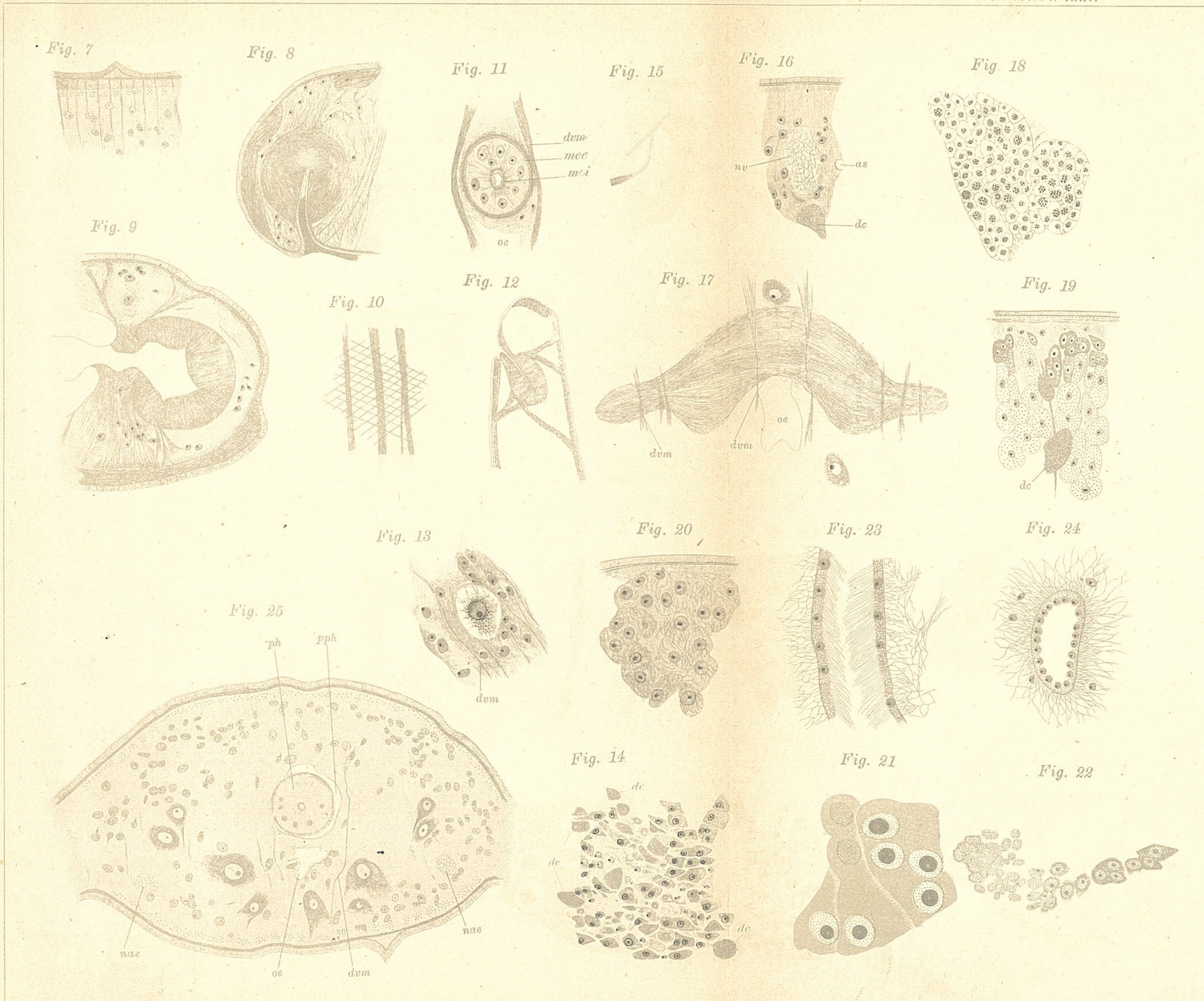
Fig. 7
Fig. 8
Fig. 11
dvm
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Fig. 15
Fig. 16
nv
as
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Fig. 18
Fig. 9
Fig. 10
Fig. 12
Fig. 17
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dvm
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Fig. 19
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Fig. 13
dvm
Fig. 20
Fig. 23
Fig. 24
Fig. 25
ph
pph
nae
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dvm
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Fig. 14
dc
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dc
Fig. 21
Fig. 22


I
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S. Goto del.