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On two new Amphistome parasites of Sumatran fishes.

(Voyage of Dr. W. Volz.)

By

Dr. W. G. MacCallum, Assoc. Prof. of Pathology.

(The Johns Hopkins University, Baltimore.)

With 2 figures in text.

It is difficult to find any satisfactory literature bearing upon the trematode parasites of fish which fall into the family of the Amphistomidae or as Fischoeder has recently named them, the Paramphistomidae. A search of the references given in v. Linstow's Compendium and in the catalogue of the Bureau of Animal Industries in Washington has resulted in my finding descriptions of a few forms only. These were made by Diesing 1) from the preserved material collected by Natterer in his voyage through Brazil and later copied by Dujardin and others. The descriptions, however, and even Diesing's illustrations are so entirely lacking in anatomical details that it is impossible to say whether the forms which he observed were or were not similar to those which we have before

¹⁾ DIESING, Monographie der Gattung Amphistoma und Diplodiscus, in: Ann. Wien. Mus., Vol. 1, 1836.

us for study — the size and the form of the posterior sucker being practically all upon which one could base such a comparison. These forms were A. oxycephalum from Salmo and Silurus, A. megacotyle from Silurus, A. cylindricum from Callichthys, A. cornu and A. ferrum-equinum, also from Callichthys, and A. attenuatum from Salmo. Since this time there have appeared as far as I can ascertain no descriptions of new amphistome parasites of fishes.

FISCHOEDER 1) has, however, recently reviewed in a very complete manner the related forms which are found in mammals and this systematic study throws much new light upon those parasitic in the lower animals. In his tabulation and classification of the members of the group he introduces many new generic and subgeneric names which on the whole seem well justified, the chief division into two great groups depending largely upon the presence or absence of pouch-like appendages to the anterior or mouth sucker. Those possessing these appendages he distinguishes from the Paramphistominae under the name Cladorchinae with the genera Cladorchis, Stichorchis, etc. Both the worms which have been kindly referred to me for study by Dr. Volz possess these saccular appendages about the anterior sucker, and therefore fall into the subfamily Cladorchinae. We may reserve a further discussion of their systematic position until a description of the worms themselves has been given.

Cladorchis pangasii n. sp.

A number of specimens of this stout, rounded trematode worm were obtained from the intestines of Pangasius nasutus Bekr., a Siluroid fish which feeds upon the fallen fruit of a mangrove tree. They were collected in the Residency Palembang (Sumatra). These worms vary a good deal in size the larger measuring 6×3.1 mm while there are specimens which reach a length of only 3.5 mm and a breadth of 2 mm. They are almost elliptical in form, flattened, but still quite thick with thick rounded edges. The formalin specimens have a pale, greenish-grey color and are somewhat translucent. Most of them are curved ventrally in the longitudinal direction but not hollowed ventrally from side to side. Anteriorly there is to be seen a small opening surrounded by a muscular mass of small size.

¹⁾ FISCHOEDER, Die Paramphistomiden der Säugethiere, in: Zool. Anz., Vol. 24, 1901, p. 367. — Desgl. in: Zool. Jahrb., Vol. 17, Syst. 1903.

At the extreme posterior end there is a large sucker which in the average individual measures 1,2 mm in breadth, the aperture measuring 4 mm. There is on the ventral surface at a point 0,8 mm behind the mouth in the median line the aperture of the genital cloaca. Otherwise the skin is quite tense and smooth except for the orifice of the excretory apparatus which lies just in front of the posterior sucker in the median line dorsally, and the minuter orifice of Laurer's canal which may be seen a short way further forward also in the median dorsal line. The skin shows a fine network of anastomosing lines, apparently cuticular canals. which unite on the ventral surface into four trunks one at each margin, and one on each side of the midline ventrally about half way out to the margin. These converge anteriorly about the mouth. About the posterior sucker these canals are especially abundant. There is no armature of cuticular spines. The orifice of the mouth is quite small being terminal and surrounded by concentric folds of skin but not showing externally any definite sucker-like arrangement. There is, however, a moderately strong sucker which is somewhat deeply embedded in the tissue and which is particularly characteristic in that it possesses two lateral muscular pouches. These are about as large as the sucker itself and have quite the same muscular structure. It seems, therefore, most reasonable to regard them as sucking pouches although Walter in describing *Diplodiscus sub*clavatus, interprets them as salivary glands. These pouches lie dorsally and between and below them there lies the oesophagus which opens backward from the mouth cavity. This is short giving rise almost at once to the curious elongated muscular pharynx. In this the alimentary canal is narrowed to a very fine epithelium-lined tube which is surrounded by many dense concentric layers of muscular or elastic tissue. Outside this there are abundant cells closely applied to the concentric lamellae. Most of them are rounded or cuboidal or irregular nucleated masses, but there are some large vacuolated cells with large vesicular nuclei which look almost like ganglion cells. At about the level of the genital cloaca the pharynx goes over into the intestine proper which divides into two lateral coeca which run toward the posterior part of the body and end blindly a short way in front of the posterior sucker. While the pharynx is lined with cuticle the intestinal coeca have a lining of cubical epithelial cells which are, however, provided with long cilia so uniform in length and so thickly matted together that the mucosa

of the coeca appears extraordinarily thick and the lumen extremely narrow. There is a quite thick muscular coat of circular and longitudinal fibres and outside these there are numerous irregular cells accompanying the coeca and sending processes between the muscle bundles.

The excretory system in this form is interesting in its arrangement, but it is so closely associated with the parenchyma that that must be first described. The worm is quite translucent and withal quite thick dorsoventrally. The translucency is seen on section to depend largely on the structure of the parenchyma. This is composed of ramifying cells whose nuclei are very inconspicuous and small. The cell body is also rather attenuated, the main bulk of the tissue being made up of great spaces between the cells which are filled with a coagulable fluid. This fluid is not everywhere of the same consistence for while in some spaces it is slightly granular and stains scarcely at all. in others it becomes more dense and assumes a pink stain. Finally there are several canal-like spaces. two on each side, dorsal and ventral to the intestinal coeca, which run throughout a large part of the length of the body and which contain an especially dense colloid-like, pink-staining fluid. These canals seem to be actually lined by an endothelium-like layer of flat cells but it does not seem possible to outline them clearly everywhere, and one receives the impression that they communicate with the other spaces between the cells. Such canals or lacunae surround the anterior and posterior suckers which seem to be suspended in them.

Beginning at the extreme anterior end of the body there are several canals with basement membrane and much convoluted wall lined by high cubical or columnar or even bulbous cells which carry a great deal of brown pigment. There are three of these canals on each side anteriorly, but as they approach the posterior end they unite into one canal on each side and eventually these trunks approach the middle line and joining to form a thicker tube whose wall is even provided with muscular fibres, the system finally opens at the point in the median dorsal line just in front of the posterior suckers, referred to above. This system of pigmented canals which can be seen in the whole worm by transmitted light must be regarded as the excretory or water vascular system. Cf. Fig. A (page 674) in which only the pigmented canals and the median excretory sac are represented.

Now in sections it is seen that the canals filled with the red staining colloid-like material stand in direct communication with these pigmented trunks. On can here an there find the pigmented canal almost suspended in the lumen of the other. This seems to depend merely on the proximity of one to the other, but in addition there are distinct canals which form a direct communication between the endothelial tubes and those with pigmented epithelium. In these hardened specimens no ciliated funnels could be recognized, but it seems evident that the excretory system as described comes into very intimate relations with the parenchyma.

The nervous system has not been thoroughly studied. It consists, however, of lateral ganglia near the oesophagus joined by a commissure which passes over the buccal pouches. This is very thick and many ganglion cells may be seen associated with it and the ganglia. Trunks run, as usual, forward and backward along the sides of the body. It is curious that there are to be found especially in association with the nervous system, but also scattered through the parenchyma and chiefly just beneath the skin, and about the alimentary tract, abundant, irregular cell-like structures loaded with eosinophile granulations. These vary in size and form, and it is usually very difficult to make out anything resembling a nucleus. Their exact nature and function I must leave obscure.

Other cells, the well-known subcuticular cells are present in quantities among the cuticular fibres sending their processes through to the skin.

The worm is, of course, hermaphrodite. There are two welldefined, lobulated testes situated at about the middle of the body. one in front of the other. They have not fused into one mass as has been described for members of this genus. On the contrary, each sends a stout vas deferens from its dorsal anterior portion to the elongated and convoluted cirrus sac. The sac into which these canals open is very thin-walled, very long, and much folded upon itself, and is usually filled with spermatozoa. It communicates directly with the convoluted thick-walled but narrow tube which lies inside the actual cirrus sac and which opens at the point of the papilla which lies in the genital cloaca. This tube is lined with thick, rough cuticle and is provided with a concentric coat of what appears to be muscular tissue. In the terminal portion of the sac it is surrounded by a mass of large, irregular cells with large vesicular nuclei outside of which there is finally the thick. fibrous wall of the sac itself. In all probability this ejaculatory apparatus is not protruded to any great distance from the cloaca.

The ovary is situated rather toward the ventral surface behind the testes and a little to the right. It measures about 0.26 mm in diameter and is rounded or pear-shaped. Dorsally it gives off a short oviduct which is quickly joined by the thick-walled, narrow Laurer's canal which opens as stated above in the midline dorsally. The tube produced by their union passes into a mass of large cells with large vesicular nuclei, a tissue which is doubtless to be interpreted as a shell gland. There it is joined by the wide canals which lead on each side from the laterally placed vitellarium and from this point there begins the canal, at first thick-walled and narrow, later quite wide and thin-walled which is the uterus. The uterus shows great variation in width and in the thickness of its walls, for while some convolutions are packed with eggs and granular or filamentous material there are other portions which are empty and contracted so that the wall is quite thick. The convolutions are most tortuous in the posterior part of the body behind the testes and there most of the eggs are to be seen. The eggs are elliptical. quite smooth, and thin-walled, and measure about 0.145×0.080 mm. They are usually present in rather small number. The vitellarium consists of lateral lobulated masses which extend on each side from the level of the bifurcation of the intestine to the end of its coeca. For the most part, the lobules contain large clear cells with relatively small, darkly-staining nucleus, but in the portions immediately about the transverse ducts, the cells become filled with large granules. The uterus passes forward dorsal to the testes to open also at the termination of the papilla in the genital cloaca posterior to the opening of the ejaculatory duct. The terminal portion is lined by cuticle.

Of Diesing's forms only Amplistoma oxycephalium from Salmo and Silurus and A. cylindricum from Callichthys are at all similar to this. It is true that both of these are also parasites in the intestines of Siluroid fishes in fresh water tropical streams, but while they were collected in Brazil the other form was found in Sumatra.

His descriptions are briefly as follows:

A. oxycephalum. A. corpore polymorpho, planiusculo, compresso ant tereti, oblongo ovato, aut ovato lanccolato. ore terminali orbiculari; acetabuli suctorii lateralis orbicularis hine postice sinuati limbo prominulo. The worm varies in size and form, measuring 2—6 lines (4.5—13.5 mm) by 1—3 lines (2.25—6,75 mm). The body flat or compressed, long, oval or lanceolate, brownish or white. A network is often seen in

the skin. The terminal round mouth is sometimes surrounded by an elevated margin. Sucker is at the margin of the tail end. It is circular, but sometimes projects backward with a swollen margin. An eminence behind the mouth carries the genital opening. The illustrations as far as they go, except for the lancet form and pointed head and the irregularity and projection of the posterior sucker. might well represent the form under consideration.

A. cylindricum. A. corpore cylindrico, utrinque obtuso, transversim rugoso, ore terminali orbiculari prominulo; acetabuli suctorii orbicularis lateralis limbo elevato. Measures 4 lines (9 mm) by 2 lines (4,5 mm) white, body cylindrical rounded at the ends, transversaly wrinkled, mouth terminal, circular with elevated margin. Circular sucker lies just anterior to the posterior and with a broad margin. The illustration shows merely the outline. There is nothing in this description with is inconsistent with that of our form, nor is there anything which could insure their identity.

On the whole, notwithstanding the close relation that must exist between these forms, it seems best on account of its different host and the slight differences which do exist, to give this worm a specific name. Possibly if Diesing's material is still in existence a detailed examination of his forms may make clear the relation. A brief anatomical description for diagnosis may be given as follows:

Cladorchis pangusi.

Body elliptical, flattened with thick, rounded edges, measures 6-3,5×3,1-2 mm. Anterior or mouth sucker small, posterior sucker round, not sinuous, embedded ventrally, just subterminal. Ovary anterior to posterior sucker; Laurer's canal opens medially in front of median excretory outlet; testes lobulated, situated anterior to ovary one in front of the other; pharynx club-shaped, muscular; small lateral buccal pouches: pigmented branches of excretory system, probably connected with the network of tubules in the skin.

Cladorchis helostomatis n. sp.

The second bottle contained two specimens of a small trematode, one of which was cut into sagittal sections, after being compared with the other.

The worms were found in the stomach of *Helostoma temmineki* (K. et v. H.) which is a labyrinth fish also collected in Palembang. Sumatra

They are about 3 mm in length and 1,5 mm in breadth at the widest part and of a yellowish-brown color as they lie in the formalin solution. The posterior sucker, as may be seen in the sketch, is the most conspicuous feature, being especially large and

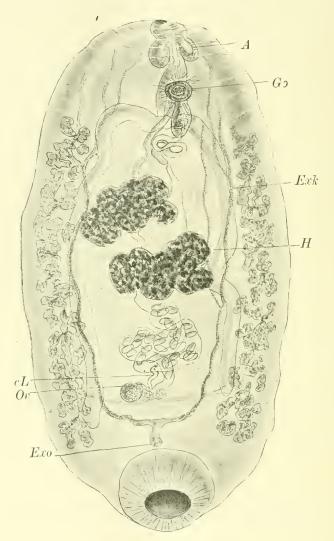


Fig. A.

Cladorchis pangasii n. sp. Ventral surface. A buccal pouch. Go genital cloaca. Exk excretory canal with pigmented walls. H testes. Lc Laurer's canal. Oc ovary. Exo excretory orifice.

characterised by its curious, sinuous, puckered outline, three spoutlike projections extending out backward. The sucker is capacious, rather thin-walled, and bounded in front by the hood-like anterior margin. The worms have been fixed in such a position that in one the dorsal surface is concave and the head end therefore turned

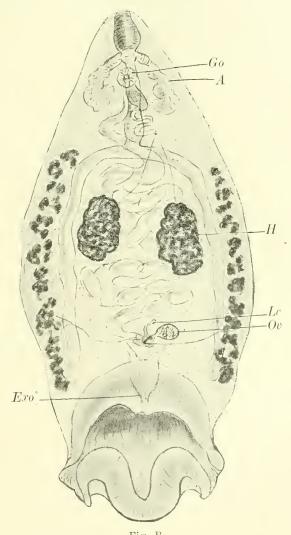


Fig. B.

Cladorchis helostomatis n. sp. from ventral surface.

Lettering as in Fig. A.

upward, in the other, the ventral surface is concave. The margin of the body on each side is somewhat thinned out and forms a projecting edge. The details of the anatomy worked out from the serial sections are as follows:

The mouth, which lies at the anterior end, opens into an elongated sucker. The genital cloaca surrounded by a sucker-like muscular arrangement is situated on the ventral surface a very short distance behind the mouth sucker separated from it by about the length of the mouth sucker.

The muscular mouth sucker opens into a sort of prepharynx of similar structure which, in turn, communicates with an elongated club-shaped muscular pharynx. On each side the prepharynx gives off a short muscular tube with circular and radial muscle fibres and many gland cells which extend into a blind convoluted sac lying on each side beside the pharynx. These lateral sacs are lined by a thin cuticle and have an inner circular layer of muscle as well as the loosely arranged radial layer the fibres of which run out to a thin outer membrane. The short tubes connecting them with the prepharynx and thus with the mouth sucker are lined with thick cuticle and have several layers of circular fibres as well as the radial fibres. This seems in this instance to be a curiously elaborate development of the lateral pouches from the mouth sucker and it is difficult to understand what function they can fill at such a distance for they could hardly aid much in the suctorial activity of the mouth parts when separated from the mouth by the narrow contractile tube described. The long, club-shaped channel from the prepharynx to the intestine which opens almost directly into the intestine is lined with a thick cuticle. It is relatively thin anteriorly but strengthened posteriorly by a thick circular layer of dense pink-staining bands in which one sees no nuclei and which are possibly muscular in nature. Outside this there is a membrane and some loose tissue containing large cells with large vesicular nuclei and nucleoli.

There may be some question as to whether this thick muscular portion of the oesophagus should be regarded as a pharynx or merely as a peculiarly thick oesophageal wall. In forms in which there is also a sharply differentiated muscular structure between the mouth sucker and the intestine this question would hardly arise. Here however the thick oesophageal wall corresponds at least in function with the pharynx of other trematodes.

The intestinal coeca are simple and run back on each side to the anterior level of the large sucker. They are lined with rather high columnar cells which are covered with a thick layer of some homogeneous material.

The posterior sucker is rather thin-walled and has the usual muscular structure.

Just in front of it is the ovary which gives off the oviduct dorsally to a mass of cells, probably the shell gland, from which the Laurer's canal runs backward dorsally to the median line. The vitellarium lying on each side is composed of rather coarse lobules of tissue and extends forward almost to the posterior level of the buccal pouches and backward to a point a short distance in front of the posterior sucker. It sends a duct from each side to the oviduct where it passes the shellgland. Thence the uterus in many coils passes forward, the coils being seen in front of the bifurcation of the intestine. It opens by a narrow canal in the genital cloaca on the ventral surface. The eggs are numerous and large, measuring 0.145×0.064 mm.

The testes are rounded and little, if at all, lobulated. They lie side by side at about the juncture of the anterior and middle thirds of the body. Each gives off a vas deferens which runs to the long, thin-walled sac which opens into the thick-walled cirrus which is evidently protrusible through the outlet of the muscular genital cloaca.

The excretory system, as far as discernible, consists of a thinwalled muscular excretory sac which opens dorsally directly over the posterior sucker in the median line. Anteriorly it branches and the branches are deeply pigmented, in this point resembling those of the preceding form.

The only one of Diesing's forms which could possibly be compared with this is A. attenuatum, which is, however, larger $(3,7-4,5 \times 2,25 \text{ mm})$ and tapers posteriorly to an extremity provided with an oval, elongated sucker. This form was found in a species of Salmo. From these differences, the description giving no particular points of resemblance. I have no hesitation in concluding that we have a hitherto undescribed species, and naming it accordingly.

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Cladorchis helostomatis n. sp.

Body truncated posteriorly, narrowed anteriorly, measurements 3×1.5 mm, large posterior sucker with sinuous margins; ventral genital opening anteriorly placed near mouth-sucker and provided with muscular ring. Laurer's canal opens medially in front of median excretory opening. Testes side by side, little or not at all lobulated. Oesophagus club-shaped, muscular, buccal pouches very large and convoluted.

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