A NEW BLOOD FLUKE FROM TURTLES*

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For several years an interesting trematode has been under observation in the laboratory here. It occurs in various species of turtles, and was first discovered in some material shipped in from the south for class work. Peculiar importance attaches to the fact that it is a species inhabiting the circulatory system, and in fact it shows a relationship to the blood-inhabiting flukes of man which has become more clearly evident as the observations have accumulated. Since the material is easily obtained, it will afford perhaps the best opportunity available in this country for the laboratory study of forms adapted to this peculiar environment, so that, despite the incompleteness of the observations, the publication of this note is justified. It is further called for by the fact that several others, who had their attention called to this species, plan to give it a more detailed study than I can make at the present time, and will be glad to have a record of the facts thus far determined in order to utilize them as a basis for further study.

For this very unique species I propose the name *Proparorchis artericola* gen. et spec. nov.

The parasite has been found in several distinct species of turtle from widely separate localities. Thus, according to records of the collection here, it has been met with in *Pseudemys elegans* from Havana, Illinois, in *Malacoclemmys leseuerii* from Newton, Texas, in *Pseudemys scripta* from Raleigh, N. C., and in *Chrysemys marginata* from Fairport, Iowa.

OBSERVATIONS ON LIVING MATERIAL

The general distribution of the parasite in the body of the host is well illustrated by the record of one very careful examination made in May, 1915. The specimen was *Pseudemys scripta*. The circulatory system was first studied and the examination of a large quantity of blood gave only negative results. After ligating veins and arteries, the heart was removed and four flukes found in it. Several large veins were taken out and teased, but no flukes obtained. When, however, the large arteries were subjected to similar treatment, three flukes were taken; one was found plugging up the end of an artery. Both lungs were teased out; one yielded three flukes, the other none. Negative results came from similar handling of the liver. All organs examined

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contained eggs. A large number were present in the brain. They seemed to be more numerous in the lungs and digestive system than in the muscles.

A year later another turtle of the same species was examined with almost identical results. Every precaution was taken to prevent transfer of material from one organ to another or loss of any during the examination. The heart contained four large flukes, the arteries three, and the veins none. One lung yielded three flukes and the other none. The intestine and mesentery were stretched out and fixed in that condition. A methodical examination revealed no flukes in these organs altho eggs were very abundant in the mesenteric vessels.

When the living worms are released from blood vessels into normal salt solution, they often display marked activity and swim about so rapidly that it is difficult to follow them. The method of progression in the fluid resembles that of leeches and is sufficiently powerful to convince the observer that they can probably make progress against the blood stream in the arterial circulation. Their orientation in respect to the direction of the current in the vessels varies.

As has already been noted, they were never found in the venous circulation, but were taken in arteries and in the heart. This location was finally verified by ligating the blood vessels, removing the heart and sectioning it *in toto*. A fluke was found in the ventricle. In this host, also, it was found that eggs were very abundant in the walls of the ventricle and present tho less abundant in the walls of the auricles. The numerical difference was somewhat proportional to the thickness of the muscular tissue in these two regions. The turtle examined in this instance was rather small, and hence young. Yet it was generally infected with eggs in various tissues.

Almost all tissues contain eggs in case the turtle is generally infected but the mesentery and the lungs seem to accumulate the most. In extreme cases these organs are crowded so full that, as can be most easily observed in the case of the mesentery, the eggs serve to outline the course of the arteries. In the mesentery the ova vary widely in color and general appearance. Some are only faintly colored with a clear transparent shell that enables one to determine readily the character of the enclosed embryo. Those at the other extreme are deep brown, almost black, and entirely opaque. None of the shells had opened and apparently none of them escape from the vessels. The mesenteric arteries would thus form a graveyard in which ova would accumulate without achieving for the species their purpose.

It is interesting to compare with this an observation reported to me by one of my students. He examined a small turtle that had died after being kept some time in a laboratory tank and found the lungs filled with eggs. Many of them were empty shells from which the miracidia had escaped for the lid was open. It is of little value to speculate on the fate of these embryos. It is clear, however, that if at the time of oviposition the flukes resort to the ventricle, which they surely do visit as I have shown, then the ova will be carried in part into the systemic arteries and in part into the pulmonary arteries. The ultimate results seem to be different in the two cases. Eggs have also been obtained from the feces of infected turtles.

The number of parasites found in a single host has never been very large whereas the number of eggs was often very great. This points to the gradual accumulation of the ova in the vessels, perhaps over a considerable period. Some turtles have been examined without finding any of these flukes and yet eggs occurred abundantly in the tissues. In other cases only very young flukes were found and these had not yet begun to produce eggs altho the eggs were numerous in the mesenteric vessels. While some parasites might easily be overlooked, yet these cases indicate that the flukes which had produced the eggs, had died and the young parasites were a later infection.

Some observations were made on the eggs containing living embryos. For this purpose eggs were taken from the feces of *Chrysemys marginata* and treated with dilute solutions of HCl from 0.02 to 0.1% in strength. The effect on the miracidia was very evidently stimulating, but those that hatched out were killed by the action of the acid immediately after the rupture of the membranes, and some were killed while even yet within the unbroken egg shell. This would seem to indicate extreme sensitiveness to gastric digestion and preclude direct infection of a new host by way of the alimentary system at least.

Eggs placed in hanging drop cultures hatched out in from 4 to 24 hours after being mounted. From these eggs and also from others placed in pure water, the miracidia seemed to emerge sooner than from eggs left in feces in a petri dish. When eggs with mature embryos are broken open in normal (0.75%) salt solution, the miracidia can be studied and later preserved. Free miracidia were found in feces cultures, but all those seen there were dead. Their escape from the shell was not observed. Other eggs in the feces contained apparently fully developed miracidia that had died without hatching out. No light was obtained on the conditions controlling the normal escape of these embryos from the eggs.

The unripe embryo is rather quiet, but shortly before hatching it becomes increasingly active, first by moving parts of the body and then by rotation on its longitudinal axis. This rotation increases in rapidity and is accompanied by pronounced contractions of longitudinal and circular muscles which turn the embryo so that it may assume any position within the shell. These violent movements ultimately loosen the cap of the egg shell and tilt it to one side like a lid fastened by a hinge. The miracidium forces its way bit by bit thru the open door which is not large enough to permit its immediate exit, but once free it swims round and round in the free water with relatively great speed and energy. In all observations made here, they lived only a very short time (5 to 10 minutes), becoming rapidly distorted and ceasing all activity thereupon. It is probable accordingly that these observations did not present normal conditions for opening the eggs or for the miracidia afterwards. Of course the conditions may have brought about precocious hatching, but none of the eggs hatched which were held under observation for longer periods.

Miracidia still enclosed in the egg shell measure about 28 to 14μ whereas those free in water or feces are distinctly longer and slenderer, measuring about 30 by 11μ. Within the shell one finds a large oval globule (Fig. 7) slightly greater in dimensions than the embryo. The miracidium has a large black eye spot which always appears irregular, and in favorable circumstances shows the form of contiguous reversed crescents usually designated as X-shaped. Some large gland cells are seen faintly in the living specimen, and its surface is covered with a coating of long cilia which are comparatively thinly distributed. The anterior end carries a cap-like structure which in the free swimming miracidium (Fig. 9) becomes a small bluntly rounded conical papilla. The ducts of the glands open on the summit of this papilla.

STRUCTURE OF THE ADULT PARASITE

The adult worms, which are easily found on careful examination of the mesenteric vessels of infected turtles, are small and conspicuously transparent. In size, they measure from 1.62 by 0.28 to 2.62 by 0.77 mm. In the smallest the ovary was small and no ovum had yet developed but ripe sperm cells were found in testes and vesicle. The body is an elongated oval, or spindle shaped tapering slightly towards sharply rounded ends. The anterior end is more nearly pointed and much more mobile than the posterior. The body is relatively thin, measuring not more than 70 to 80μ in dorso-ventral diameter, and in the preserved specimen is regularly hollowed out a little on the ventral surface both longitudinally and transversely. The margins of the body are noticeably thin and sharp. In the blood vessels the worm appears to be much slenderer and longer than when observed outside the body of the host or in alcoholic specimens. The transparency of the body is due to the relatively slight development of the muscular layers which are represented only by thin sheets of very delicate fibers.

At the anterior end one notes the single sucker present in this species. It is peculiar in form, being a greatly elongated oval with relatively small sub-terminal opening. It projects forward in an unusual fashion, and imparts to the anterior end a characteristic appearance which is rarely met with among trematodes.

The surface of the body is smooth and without spines or scales. None of the small wart-like structures with fine spines have been found in this species which are described by Looss and others for other types of blood-inhabiting flukes. In preserved worms, which are somewhat contracted at the anterior end, the esophagus is slightly sinuous and the inner wall plicated. It has a relatively large lumen and increases in external diameter posteriad. The cavity varies noticeably in width, having one or two wider regions much such as are figured in the Schistosomatidae by Looss (1895, pl. 2, fig. 18). There is no evidence whatever of a pharynx, but near the posterior end (Fig. 5) the wall of the esophagus is conspicuously thickened by an accumulation of what are certainly gland cells. These take a deep stain, and while so irregular in form as to preclude the possibility of interpreting them as a muscular organ, yet superficial examination might lead one to designate this region as a pharynx. It is, however, at the very termination of the esophagus, taking in one-fifth or one-fourth of the entire length of the organ and not separated by any interval whatever from the diverging crura. These gland cells are densely crowded and in this posterior region occur in several layers so that they seem to form an enlargement of the esophageal wall. A thinner layer covers the wall of the esophagus for its entire length. Similar conditions were originally described by Leuckart for Schistosomes and fully verified by Looss. The likeness between Proparorchis and Schistosoma in respect to the esophagus is so complete that it extends even to minute details of structure. Looss (1899:751) reported similar glandular structures in Hapalotrema and denominated them salivary glands. It is thus evident that they are all but universal in blood-inhabiting flukes and indeed will probably be found in species from which they have not yet been recorded. Their development is undoubtedly due to the type of food utilized by these flukes.

The intestinal crura are markedly sinuous in outline and nearly equal in caliber throughout the entire length. They extend to within a short distance of the posterior end and there turn somewhat towards the center, although always remaining distinctly separated from each other. The cells which line them are filled with a dark granular substance, suggesting the origin of this material from the blood of the host (Cf. Looss, 1895, pl. 3, for Schistosomes). The crura diverge almost at right angles from the esophagus, forming a conspicuous cross bar and an equally conspicuous angle at the side where they turn backwards. Directly opposite the point of junction with the esophagus is a median structure which stains conspicuously and is apparently glandular. It has only in part the same appearance as the crura themselves, and might be regarded as a median diverticulum with a very short lumen. However, the cells are not filled with the dark granules which impart to the intestine its characteristic appearance. The lining of the diverticulum is a very thin membrane and at its base is a mass of amorphous material which resembles in appearance and in staining qualities the inner layer of the esophagus (Fig. 5).

The excretory system is easily seen at the posterior end of the body. It presents the form of a bifid bladder or perhaps of small lateral bladders connected by very short stalks to a common duct, which is equally brief and opens at the median pore. The latter is nearly terminal in location. The lateral bladders are a little shorter than the space between the posterior tip of the body and the end of the intestinal crura. Anteriorly one sees a single longitudinal vessel connected with each bladder. Further details of the system have not been worked out.

The main features of the nervous system are distinct in living specimens and also in toto-preparations. The anterior ganglion spans the esophagus a short distance back of its junction with the oral sucker. The lateral nerves are relatively heavy and can be traced the length of the body. These features are relatively larger and more conspicuous than in most flukes. Here again the conditions recall those in the Schistosomes as reported by Looss (1895).

The most striking feature in this parasite is the peculiar development of the reproductive system. The organs are nearly all confined to the area within the intestinal crura. The testes (Fig. 1, *t*) occupy the major portion of the space anteriorly. They begin a short distance behind the fork of the intestine and extend as a series of irregularly lobed bodies down the median line a distance equal to about one-half of the entire length of the worm. In this group are from six to ten or more irregularly shaped bodies, more or less flattened on the anterior and posterior faces by mutual pressure but deeply lobed on the lateral aspects. In many cases it looks as if the parts were continuous, but sections show well developed limiting membranes separating them. It may be that there is a fixed number of separate parts in this testicular area but the varying stages of contraction in different specimens make it difficult to reach a positive conclusion. Immediately behind the posterior testis is a seminal vesicle (Fig. 3, *sv*) which is elongated, pyriform and connects directly with the cirrus (*c*). No distinct prostate cells were seen and both the cirrus and the cirrus sac are delicate and difficult to detect. The pyriform vesicle and the duct form a nearly straight passageway from the center of the posterior testis to the common genital pore (*gp*). This opening is located about on the level of the intestine at the left side and ventral.

The ovary (*ov*) is a many-lobed structure in the intracrural area behind the testes; it lies chiefly dorsal. The vesicle and cirrus cross ventrally the left ovarian lobes. The opposite face of the organ is pressed closely against the intestine on the other side of the body. The yolk glands (Fig. 3, *v*) are exceedingly voluminous. They begin about at the end of the esophagus and extend just a little beyond the posterior ends of the intestinal crura. The cells tho not crowded form an almost continuous strip or band which lies below and, to some extent, on both sides of the crura but only in the immediate proximity of those structures, for the central area of the body is entirely without yolk cells. At the end of the esophagus and behind the crura, the cells from the two sides approach and become confluent in the median line. Behind the ovary on the ventral side of the body, the transverse yolk duct joins the two yolk glands and on it in the median line is formed a prominent yolk reservoir (*yr*). There are no reproductive organs behind this limit, except some of the outlying cells of the vitellaria already mentioned.

The ducts of the female system are strikingly simple, and are crowded together in a small triangle between the ovary, the cirrus, and the transverse yolk duct. The relation of the different structures will be apparent from the illustrations (Figs. 2, 3) one of which represents a reconstruction of this area from a series of sections. One can readily identify the various structures. A small expansion on the oviduct near the ovary is seen to be the receptaculum seminis uterinum (*rsu*) which is easily recognized by the considerable mass of sperm cells that it contains. After a brief course dextrad and posteriad the oviduct turns sharply back on itself near the intestine and swings in a crescentic curve to the sexual pore on the opposite side of the body. About at the angle made by this turn, there is given off a short tube which mounts almost directly to the dorsal surface. This is Laurer's canal (*lc*). It is relatively large and open, and contained ripe sperm cells in those specimens which were sectioned. Half way from this point to the genital pore, the canal is slightly expanded, and in this expansion lies in many specimens a single egg. This tube corresponds in position and connections to the long convoluted uterus in most flukes, but instead of carrying a mass of eggs such as is usually found in that organ, it never contains in this form more than a single one. The short stretch which intervenes between this region and the pore is distinctly provided with a muscular layer in the wall (Fig. 4). This region is the metraterm; the egg lies really in the ootype and a true uterus is lacking.

The eggs outside of the worm in the blood-vessels of the mesentery as already noted are in part light colored and semi-transparent, and in part dark brown and almost opaque. The latter seem to be the older eggs. Sets of eggs from different places were measured, and the results are given in the following summary. All measurements are given in microns.

Preparation, No.	Number Measured	Average Length	Average Breadth	Length Max.	Length Min.	Breadth Max.	Breadth Min.
15.54	19	95.9	75.5	105.6	70.4	96.8	61.8
15.71	20	103.4	77.4	123.2	88.0	88.0	70.4
15.71	20	106.5	78.7	124.2	81.0	94.5	64.8
15.72	20	110.4	81.7	121.5	97.2	91.8	75.6
15.72 *l*	12	101.2	82.7	114.4	70.4	96.8	52.8
15.72 *d*	20	95.9	77.0	114.4	79.2	88.0	52.8
General average..........		102.2	78.8				

l = light eggs only; *d* = dark eggs.

Most eggs come within the limits of 88 to 114μ in length and 70 to 88μ in breadth.

When these figures, which are obtained from preserved material, are compared with those giving dimensions of the eggs under other conditions, the results are rather extraordinary. In living specimens eggs from cultures, drawn and measured were 85x68 μ, 97x80 μ, 80.5x73·μ, 85x74 μ, 97x80 μ. In worms which had been preserved, stained and mounted *in toto,* the eggs still contained in the uterus of the female showed dimensions of 75x45 μ and 84x41 μ. The eggs from cultures and especially those still retained in the body of the female are thus smaller than the average of those found free in the blood-vessels of the mesentery. Further, eggs in the body of the worm are clearly oval, whereas those outside are more nearly spherical. Looss (1902:522) noted also that the egg shell may increase in size during the growth of the embryo; this was observed in a blood-inhabiting species the adult of which was not identified.

The eggs found in various organs occasionally show stages in cleavage or much more often two black eye spots indicating that the miracidia are well developed. One encounters also many eggs in which the enclosed embryos are dead and undergoing disintegration. One of my assistants at one time found a mass of eggs in the intestine of a turtle but no evidence was secured on the method by which the eggs escaped from the vascular system or the place at which such escape was made; and the discovery noted may have been based on some sort of accidental transfer of the eggs to the intestinal contents. Nevertheless there is no doubt that eggs occur regularly in the feces of the turtles for they have been collected and studied frequently and those found there contain living embryos.

The data just given on the structure of the parasite may be summarized in the form of a generic description as follows:

PROPARORCHIS Nov. Gen.

Small trematodes with delicate body, widest at center and tapering towards both ends. Oral sucker elongate, protruding; no other sucker present. Esophagus with wide lumen, without pharynx, covered with glandular cells, prominent near posterior end; crura long, sinuous, extending nearly entire length of body. Median glandular diverticulum opposite end of esophagus. Excretory bladder double, short; excretory pore single, subterminal. Genital pore sinistral, ventral, in posterior region. Cirrus sac with slender cirrus and ductus ejaculatorius; seminal vesicle large, pyriform. Testes numerous, irregular, lobed, in intercrural area, between intestinal fork and ovarian complex. Ovary lobed, posterior to testes, chiefly on right side of body; oviduct short, with sperm filled expansion (receptaculum seminis uterinum). Laurer's canal present but no receptaculum seminis. Vitellaria well developed, conspicuous laterally, enveloping intestinal crura on lateral, dorsal and median aspects thruout entire length. Transverse yolk duct with median reservoir between ovary and end of crura. No true uterus present, metraterm extends straight from ootype to pore. Eggs deposited so soon after formation that never more than a single one is seen in the fluke. Egg provided with cap; those in body of worm measure about 80 by 45 μ, in blood vessels of host about 100 by 80 μ. Cleavage well advanced before oviposition; well developed miracidia with conspicuous eye spots in eggs taken from blood vessels of host.

Type and only species: *Proparorchis artericola* from various fresh water turtles.

The data in my possession are not all referable to the single species which has just been described. In details of structure, in regard to the eggs, in the location in the host in which they have been observed, and in some other details, certain specimens differ so distinctly from the account above that I can not at present include them under the same heading. It is possible that they represent different phases in the life cycle of a single species. I am inclined to think the structure of this worm too delicate for one to consider it probable that any part of its life history could be passed in the intestine. But such a transfer must still be kept in mind as a possibility. In my opinion it is much more likely that further study will disclose the presence of several species parasitic in the blood of reptiles and amphibia. I have myself a single specimen of a distome unlike any genus yet described which was found in the course of explorations for the species just described.

RELATED SPECIES OF FLUKES

In a recent paper (G. A. MacCallum, 1919) has given a brief description of an unusual worm found in the *intestine* of a wood turtle at the New York Aquarium. This form is undoubtedly closely related to that described in this paper. To this form MacCallum gave the generic name, *Spirorchis,* but omitted to add any specific designation. In order to insure accuracy of reference, I would suggest that his species be designated *Spirorchis innominata.* MacCallum's description is brief and in some details confused since the dimensions given are clearly wrong and the text does not agree in full with the illustration. On the other hand no one can consider his account without being impressed by the general likeness his species has to that described here. It is important to consider in a comparative fashion the structure of these two forms as a basis for a decision as to the degree of their relationship.

MacCallum's fluke is considerably larger tho of the same general form and apparently also similarly delicate in structure and transparent. The general plan of the organs is much like that in the species just described. The peculiar form and position of the oral sucker in Proparorchis is both described in MacCallum's text and shown in the figure accompanying it; on the other hand, the characteristic angle of the esophagus and crura in this form is not shown or mentioned by MacCallum, and the pharnyx which he describes and pictures near the center of the esophagus is certainly not present at all in the blood fluke I have studied. Many further items in MacCallum's account, like the appearance of the testes and of the seminal vesicle, the size and form of the ovary and of the egg, and the various measurements of the body which do not agree with the description given above, may perhaps be explained as specific differences tho they are not discussed in sufficient detail to make this opinion positive. His very definite statement concerning the location of the genital pore shows a striking difference from the condition in the species described here.

MacCallum states that his parasites were taken from the intestine of a wood turtle *(Chelopus insculptus)* but adds "as will be seen by the color of the contents of its intestines, it is a hematophagic trematode." It is not unlikely that its presence in the intestine was accidental, the result of opening some blood vessel during the dissection, and that in fact that species also is normally an inhabitant of the vascular system. But this is only a tentative opinion.

The converse of that proposition is entirely untenable. One can not maintain the view that the worms I found in the blood vessels are immature forms which might later attain the adult condition in some other location. Several conditions militate against such an explanation. First, large masses of ripe sperm cells are found in the worms, and occur not only in the seminal vesicle, but also in that portion of the oviduct often termed the receptaculum seminis uterinum. This shows that impregnation has already taken place altho, of course, this might have been self-impregnation which has been observed in encysted trematodes. Secondly, many if not all of the flukes contained in the uterus a single egg which was well started in development. Third, careful microscopic inspection of the ovary and testes gave evidence that these organs in some cases had been functioning for some time. Fourth, the blood vessels contained large numbers of egg shells which enclosed fully developed embryos; these were removed from the vessels and watched in many instances until the miracidia hatched out and swam about in the culture medium. Fifth, all stages in advancing maturity which should be present in adult worms are actually represented in the specimens found, from the young fluke in which the female organs have not yet begun to function actively, to such as show that system at its functional apex whereas the male organs have passed their prime and are already on the decline. Proparorchis becomes fully mature in the blood vessels of the host.

I have endeavored as yet unsuccessfully to get for examination one of the specimens on which MacCallum's description is based. In the light of his description it appears to me necessary to accept his diagnosis as it stands, especially since his previous publications show great care in studying out similar parasites and accuracy in stating the results of such study. Unless the differences are to be explained away as errors in observation, they form an adequate basis for the differentiation of genera even tho these are closely related and should be included in a single subfamily. I have rewritten MacCallum's account in brief taxanomic form in order to facilitate the comparison of that species with the one I have just described.

GENUS SPIRORCHIS G. A. MAC CALLUM

Small species with smooth skin, body widest near center, tapering towards both ends. Anterior sucker small, protruding; no acetabulum present. Esophagus with pharynx near the middle; crura conspicuous because of dark granular contents, extend to near posterior end, sometimes coalescing there. Excretory pore near posterior end. Genital pore median, posterior to tips of intestinal crura. Vitellaria profuse, lateral, along entire length of intestinal crura; transverse yolk duct and median reservoir near posterior limits of yolk glands. Ovary lateral, oviduct long; one large egg measuring 100 by 50 μ,* with thick shell regularly present. Testes irregular, lobed, "in rough spiral column" occupying central region between intestinal crura and followed by large

* MacCallum says 5 to 10μ, an evident error.

conical seminal vesicle which tapers to cirrus (?). Connection of ducts and other organs not seen "on account of black intestines filling the posterior end of the worm."

The genera Proparorchis and Spirorchis are evidently members of a new subfamily to which the name Proparorchiinae may be given. The position of this sub-family deserves further consideration. Its nearest affiliations are to be found on the one hand in a fluke from sea turtles described by Looss and on the other hand in the human blood flukes, the Schistosomatidae.

The first mentioned trematode from the vascular system is *Hapalotrema constrictum* (Leared), most recently studied by Looss (1902:519-). It is a common parasite of *Thalassochelys corticuta,* a sea turtle taken on the Egyptian coast. The eggs are very striking, being large and supplied with long polar processes coiled at the tip. They also occur within the tissues. While these eggs are so conspicuously unlike those of the species described in this paper, yet they enclose an embryo said by Looss to resemble closely that of Schistosoma, as also does the embryo of this species. In general appearance and structure, like Proparorchis, these worms are delicate, thin-skinned and only weakly provided with dermal musculature. In this respect they resemble also the human blood flukes (Schistosomatidae) most strikingly.

In discussing the genus Hapalotrema, Looss (1899:656) comments on its striking similarity to the genus Schistosoma in the alimentary system, the structure of the suckers and the character of the skin. Yet in view of the marked dissimilarity in other respects, he concludes that the likeness is merely convergence due to the place and mode of life since both inhabit the blood stream and feed on the blood. The resemblance to the species reported here is even more striking. The delicate body with scantily developed musculature, the peculiar esophagus, the short female genital canal, the formation and extrusion of eggs one by one, and the well developed ciliated miracidium are all peculiar and characteristic features in which the two forms agree with each other and differ from almost all other known trematodes. While a few of these features are found in the Schistosomes, as noted above and that agreement might be explained as the result of convergence, yet a similar explanation is more difficult to apply to the longer series of structural likenesses between Hapalotrema and Proparorchis.

However, the differences between the two species deserve equal emphasis. First of all, some would list the fact that Hapalotrema is a true distome whereas Proparorchis is a monostome; but to me this is a subsidiary feature since extended studies have led me to the full acceptance of the view presented forcibly by Odhner that the monostomes do not constitute a natural group but represent an assemblage of forms derived from different families of distomes by the reduction and ultimate disappearance of the acetabulum. They are thus alike primarily only in the superficial feature that all possess but a single, anterior sucker. The organ pattern, on the other hand, brings evidence of marked differences in type and indicates clearly relationship to different groups of distomes. Odhner would accordingly classify the monostomes with those distomes to which they are most clearly allied and abolish entirely the major subdivision of Monostomata. For evident practical reasons, such a radical proposal is not likely to be adopted until knowledge of the monostomes has been greatly extended.

The oral sucker of Hapalotrema is described by Looss as flat, saucershaped, projecting above the general surface of the body and scantily developed in musculature. It appears thus as if already in course of elimination, even tho the process is only well begun and the interval that separates the species from Proparorchis is still great.

There are, however, still other and much more significant differences between the two species. In Hapalotrema, according to Looss, the excretory bladder is short and branches just behind the posterior testis.* This does not seem to be the condition in Proparorchis as described above. The genital pore in Hapalotrema lies about in the center of body in the left rather than as in Proparorchis near the posterior end. The eggs are very dissimilar in general appearance, since those of Hapalotrema are provided with two long polar processes on the shell which are spirally twisted at the end. It may be noted that this difference is of the same sort that exists between *Schistosoma haematobium* and *S. japonicum,* tho of course more extremely developed. The egg here has a cover and no such structure is shown for Looss' species.

By far the most striking difference is one that affects the general appearance of the body very radically. At first glance Hapalotrema resembles a common type of distomes; the transverse yolk duct, ovary, seminal vesicle and cirrus lie in the central area and other genitalia are grouped symmetrically about them. In Proparorchis the genital complex lies near the posterior end and the other genitalia lie almost entirely in front of it. A closer analysis shows that Hapalotrema is really of an unusual type morphologically; the two testes are fragmented instead of simple, and one fills the central area anterior to the ovarian complex whereas the other testis occupies the corresponding posterior area.

Conditions in Proparorchis as described above permit of a close comparison with those in Hapalotrema if the posterior region in the latter be reduced by failure of the posterior testis to develop and coin-

* The text says *in front of* the posterior testis, but Looss' figure shows distinctly the other relation.

cident cessation of growth in the entire posterior half of the worm. As the result of this, the ovarian complex lies just in front of the posterior end of the body. The structures in this complex and anterior to it correspond very closely to those in the same regions in Hapalotrema.

Looss (1902) found in the blood vessels of sea turtles four types of eggs easily differentiated from those of Hapalotrema for which no adults were discovered despite the most careful search. These eggs, or at least two of the varieties, are much like those of Proparorchis.

SYSTEMATIC POSITION

In a most interesting and suggestive paper on the Phylogeny of the Bilharzia type, Odhner (1912) established a new family, Harmostomidae, to include the subfamily Harmostominae already worked out by Looss (1899:651-) for a series of genera he had studied, and a second new sub-family Liolopinae in which Odhner includes Liolope Cohn 1902, Helicotrema n.g., and Hapalotrema Looss 1899. The Harmostomidae are placed close to the Schistosomes, which latter in the opinion of Odhner are "with absolute certainty" derived from the former. In my opinion the family Harmostomidae and the sub-family Liolopinae are both unnatural in one and the same particular: the inclusion of the genus Hapalotrema; for this form necessitates a series of exceptions in the descriptions that really do violence to the morphological basis on which those sub-divisions are built. Hapalotrema is unlike all other species in those two groups in the presence of a long esophagus, in the absence of a pharynx, in the form of the ovary and testes, in the absence of a true uterus, and finally in the character of the excretory system which is a most fundamental feature. Hapalotrema must be removed from Odhner's sub-family Liolopinae; it forms with the Proparorchiinae naturally a new family which may be characterized as follows:

FAMILY PROPARORCHIIDAE

Delicate blood inhabiting flukes, with slender, non-muscular body and one or two weak suckers. Testes lobed, multiple, anterior (and sometimes also posterior) to ovarian complex. Laurer's canal present. Ovary lobed; no true uterus; eggs large, thick-shelled, discharged singly.

These forms are certainly related to the human blood flukes, Schistosomatidae, altho not so highly specialized for life in the circulatory system. Finally mention must be made of the peculiar blood-inhabiting fish parasites Aporocotyle and Sanguinicola which have been so thoroly studied and described by Odhner (1911.) These genera show evident morphological likeness to the forms discussed in this paper, and one is fully justified in associating relatively closely the families Aporocotylidae and Proparorchiidae.

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* Dated 1918 but not distributed until 1919.

Explanation of Plate

c, cirrus; *e*, egg; *gp*, genital pore; *i*, intestine; *lc*, Laurer's canal; *od*, oviduct; *ov*, ovary; *rsu*, receptaculum seminis uterinum; *sv*, seminal vesicle; *t*, testis; *vi*, vitellaria; *yr*, yolk reservoir.

Fig. 1.—*Proparorchis artericola*. Toto mount viewed from ventral surface. Extent of vitellaria shown by dotted line. × 40.

Fig. 2.—Posterior end of same specimen. × 70.

Fig. 3.—Reproductive organs; reconstruction from sections. × 65.

Fig. 4.—Organs near genital pore, showing cirrus partly extended, metraterm, and egg just being formed. × 270.

Fig. 5.—Frontal section of esophagus, bifurcation of crura and diverticulum. × 170.

Fig. 6.—Egg in cleavage from ducts of fluke shown in Figure 1. × 210.

Fig. 7.—Miracidium, viewed in profile, in egg shell from culture; *x*, eye spot in dorsal aspect. × 360.

Fig. 8.—Empty egg shell, showing cap. × 360.

Fig. 9.—Miracidium just out of shell; ciliary coating omitted. × 150.

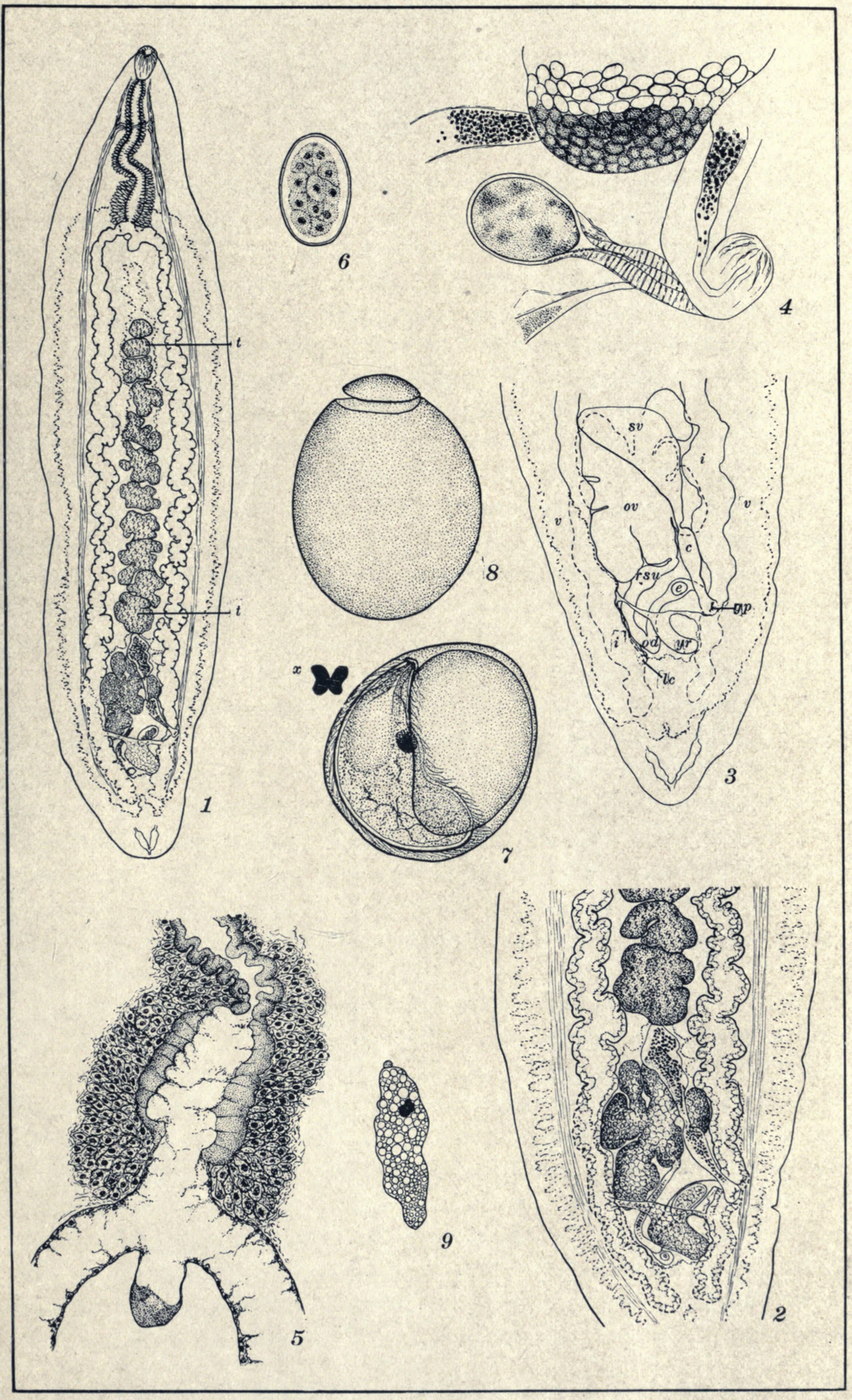

PLATE XII