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On the Metacercaria and Adult of *Clinostomum van der horsti sp. n.*, a Trematode Parasite of Fishes and Herons.

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A PRESERVED specimen of the fish *Gnathonemus macrolepidotus* was kindly placed at the disposal of the writer by Prof. C. J. van der Horst of the Zoological Department, University of the Witwatersrand. This fish had died in the aquarium of the University, and on being opened Prof. v. d. Horst noticed that it was heavily parasitized by a larval trematode. The writer identified these as the metacercariae of a clinostome, and arrangements were then made to obtain fresh fish material from Prof. v. d. Horst in order that the fresh metacercariae could be fed to a suitable host in order to obtain the adult parasite. Two live fishes were obtained, both of which proved on dissection to be heavily parasitized. These were fed to a young blackheaded Heron (*Ardea melanocephala*) which had just left the nest, and which on examination had been found to be free of helminths. The writer wishes to express his sincere thanks to Prof v.d. Horst for providing him with this material and to Dr. Bigalke of the National Zoological Gardens, Pretoria, for placing the heron at his disposal.

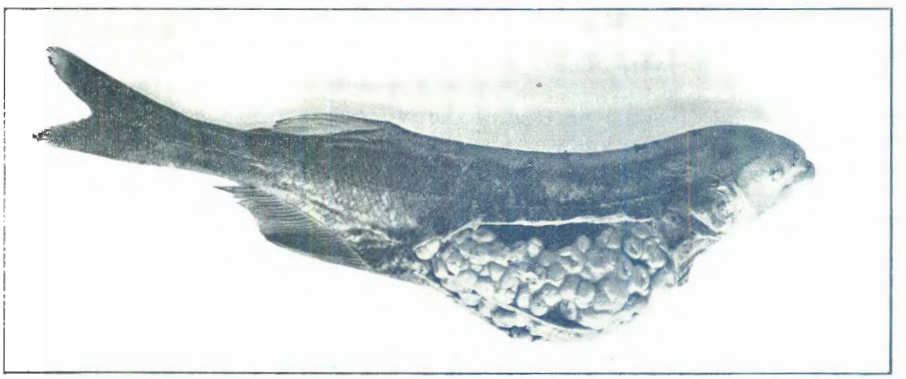

Fig. 1. *Gnathonemus macrolepidotus* (natural size) with Metacercariae of *Clinostomum van der horsti* sp. n. *in situ*.

Metacercaria.—The parasitized fishes which were examined were each found to contain about 50 metacercariae; these for the most part filled up the whole of the body cavity, being attached to the lining of the body cavity (Fig. 1). None were attached to the internal organs.

and only a few were found to be superficially buried in the muscles of the abdominal body wall. Each metacercaria was somewhat rounded, about 2 to 3 mm. in diameter, and surrounded by a thin transparent envelope; they were all lying close against each other so that collectively they looked somewhat like a bunch of grapes.

On removing the envelope it was found that the contained metacercaria was folded on itself dorsally, and when placed in normal saline it showed active movements of elongation and contraction. Some of the metacercariae were fixed according to Looss' method in Schaudin's solution, while others were examined alive under slight pressure. Serial longitudinal sections were cut of the former. As the morphology of the metacercariae is practically identical with that of the adult, it will be discussed later on. The only differences are that the metacercariae are slightly smaller, the preserved specimens being from 5 to 6 mm. long; the vitellaria have not yet made their appearance; and the genital glands are smaller.

One of the fishes containing the living metacercariae was fed to the heron by forcibly sliding the fish down the gullet. As the heron was kept in an enclosed cage there was no likelihood that it could contract an infection other than from the infected fish. It had free access to a bowl of clean tap water, and its daily food consisted of chopped up beef and mutton. The heron was unfortunately not examined until the 8th day after infection, on which date mature worms were already present; it is thus not possible to state when the adults first took up their final location. Yamaguti (1933) found the adults of *C. complanatum* (Rud) 45 hours after feeding the metacercariae to a heron. Nine of these adults were seen firmly attached to the mucous membrane of the mouth round about the glottis, and one was fixed inside the glottis. An examination of the upper half of the oesophagus revealed no further worms; as the heron had swallowed about 50 metacercariae, the proportion which attained maturity was surprisingly low.

The worms were firmly attached by both their oral and ventral suckers and it required considerable force to remove them. The ventral sucker draws a portion of the mucous membrane into its lumen and, using this attachment to anchor itself, the worm bends its head end sharply ventralwards and bores into the mucous surface with its oral extremity. There is a muscular ridge or collar just behind the mouth, and this, when pressed against the adjacent tissues, firmly fixed the head in the tissues. On being pulled the ventral anchor is the first to relinquish its hold, and a further pull frees the head which comes away with a portion of the surrounding tissues. A deep scar is left which tends to bleed profusely. During the course of the next few weeks the position of the worms was noted every day, and it was found that they change their position fairly often; the old place of fixation was always represented by a scar.

The adult worms are bright pink in colour, whereas the metacercariae are of a creamy colour. The pink colour is due to the blood on which the adults feed. As free blood was seen to be present in the vicinity of the worms, and as the head end is so firmly attached that there is little likelihood of blood escaping round the sides of the head,

it is probable that blood is pumped through the gut to be expelled at the "anus" in somewhat the same way as that described for *Ancylostomum caninum* by Wells (1931); at any rate the worms perform considerable contractile movements *in situ*, so that it would be quite easy for the blood to pass to the exterior before it had time to become digested.

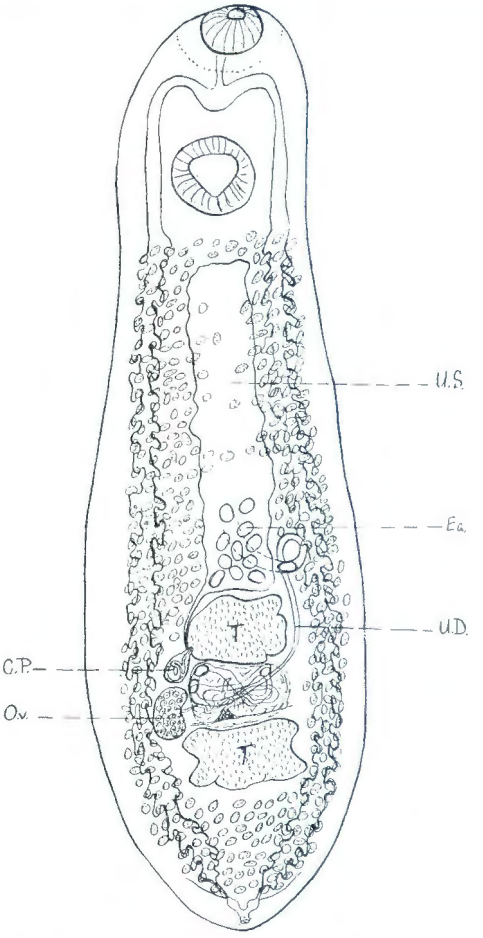

Fig. 2.—Ventral view of entire worm, drawn from living specimen slightly flattened. C.P.=cirrus pouch; Eg=eggs; Ov.=ovary; U.D.=uteroduct; U.S.=uterine sac.

Morphology.—When fixed in Schaudin's solution the metacercariae and the adults have much the same shape, except that the former is slightly smaller, being from 5 to 6 mm. long whereas the adults are from 6·25 to 7 mm. in length. The body (Fig. 2) is broadest in the region of the genital glands where it is 2 mm. broad and 0·9 mm. thick in the adult. Behind this region the body tapers rather quickly to end in a bluntly pointed tip. Anteriorly the body narrows to the level of the ventral sucker, after which the breadth remains more or less uniform to become suddenly rounded off at the

oral extremity. In transverse section the body is somewhat semicircular, having a somewhat flattened or slightly concave ventral surface and a convex dorsal surface. The cuticle is provided with very minute spines, about 0·01 mm. long; these are limited to the postacetabular region of the body and their tips hardly pierce the cuticular surface. Even in sections they are rather difficult to make out. They are present in both the metacercaria and adult.

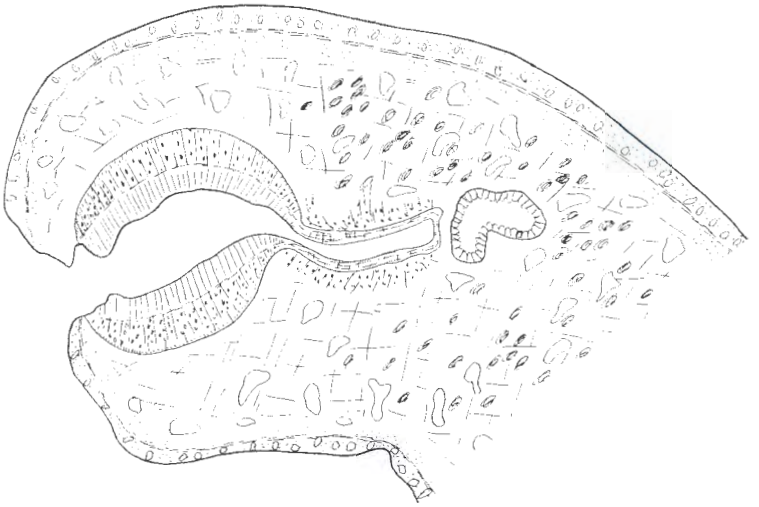

Fig. 3.—Vertical section of oral extremity showing constitution of wall of oral sucker and absence of pharynx.

The oral sucker is terminal, and its aperture lies in the centre of the somewhat flattened anterior surface; it has a slightly oblong shape, being 0·42 mm. long and 0·39 mm. broad (Fig. 3). Its wall is divisable into three parts, namely an inner purely muscular portion with radial fibres; a middle portion consisting of radial muscle fibres and glandular cells, and a thin outer portion consisting of radial muscle fibres only. A very thin layer of longitudinal muscle fibres separates the inner from the middle portion.

The ventral sucker is very muscular and is situated about 1 mm. behind the anterior extremity, in preserved specimens it is somewhat sunken into the body and its inner edge is almost adjacent to the dorsal body wall. It is about 0·9 mm. broad by 0·78 mm. long and has a depth of 0·7 mm. In living specimens its external opening is somewhat triangular with the apex pointing backwards. Its wall is very muscular 0·45 mm. thick and consists of radially arrayed muscle fibres, between which isolated rows of small nuclei are seen.

The cuticular layer varies in thickness from 0·06 mm. (metacercaria) to 0·09 in the adult. Immediately underneath it there is a very thin layer of longitudinal muscle fibres, followed by a zone of circular fibres from 0·025 to 0·047 mm. in thickness; then there is an inner layer of longitudinal fibres 0·013 to 0·022 mm. in thickness which is then followed by an inner layer of circular fibres, this layer varying in thickness from 0·032 to 0·06 mm. As pointed out

by Baer (1923) the presence of all these muscle layer in the subcuticular section is very reminiscent of that which is seen in cestodes. The parenchyma is also very well supplied with muscle fibres, circular, longitudinal and vertical, and in among these, especially on the preacetabular region, there are numerous glandular cells.

Digestive System.—The oesophagus is short and is composed of a weak muscular layer consisting of a mixture of radial and longitudinal muscle fibres; it is surrounded by a large number of glandular cells which appear to be similar to those found in the wall of the oral sucker. A pharynx is entirely absent. The two intestinal branches extend almost to the posterior extremity; from behind the level of the acetabulum each gives rise on its outer and inner surfaces to a number of conspicuous sacculations. A very small canal passes backwards from the distal end of each intestinal caecum to open into the small excretory vesicle which in its turn opens to the exterior in a fairly conspicuous pore situated subterminally and ventral (Fig. 4). That the connections between the caeca and excretory vesicle have a functional significance was noted in all the living specimens

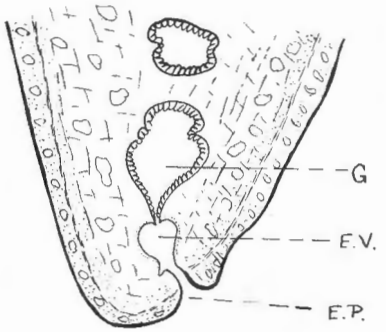

Fig. 4.—Reconstruction from four serial sections showing connection between intestinal caeca (G) and excretory vesicle (E.V.). E.P. = excretory pore.

examined, metacercariae and adults. The intestinal contents naturally flowed down these canals and passed to the exterior via the excretory pore; this could also be very easily induced when the worms were placed under coverslips under slight pressure just sufficient to make them transparent under the microscope. These connections between the caeca and excretory vesicle were also shown in section of worms which had been killed and fixed without previous examination under the microscope. The material excreted in the adult specimens consisted of blood cells in various stages of digestion.

These communications between the digestive system and excretory vesicle have recently been described and figured by Yamaguti (1933) for *Clinostomum complanatum* (Rud, 1819); although he saw in living specimens that the intestinal contents are sometimes forced into the vesicle he does not think it probable that a normal functional anus is therefore present. However, the writer, after an examination of living specimens hold a contrary view as stated above.

Excretory System.—This system consists of many branched tubules lying in the parenchyma and sub-cuticular tissues; these collect into larger tubes which pass backwards driving the excretory material backwards; one of these lies just outside of the intestinal caeca and its lumen is provided with bunches of cilia which lash backwards; these tubes join to transverse tubes just anterior of the excretory vesicle and these in turn open into two large wavy tubes, one on either side between the body margin and the longitudinal tubule described above; these large canals disappear at about the level of the ventral sucker. Except for its posterior fifth its lumen is free of cilia; these large tubes open each into one of the horns of the V-shaped excretory vesicle. The limbs of the excretory vesicle are slightly swollen and in section have a greater diameter than that portion which opens to the exterior. The excretory pore lies in the mid ventral line only very slightly removed from the posterior tip of the body.

Reproductive System.—The genital glands are situated in the posterior quarter and consists of two slightly lobed testes, between which and slightly to the right is the smaller and somewhat spherical or crescentic ovary (Fig. 1). The testes are more or less of equal size, the anterior, however, may appear smaller; they fill up most of the space between the intestinal caeca; they are about 1 mm. broad by 0·7 mm. long and 0·5 mm. thick and are about 0·4 mm. apart. The cirrus pouch is somewhat pyriform in shape, and lies just posterior of the anterior testes on its right side; it opens into the genital atrium at the level of the anterior testes. It is muscular and is about 0·6 mm. long and 0·34 to 0·37 mm. broad at its base; the cirrus is very muscular and voluminous and its surface is studded with cuticular bosses similar to those described for *C. lophophallum* Baer, 1933. A very large *vesicula seminalis* is present in the base of the cirrus pouch; it is very much coiled on itself. A *pars prostatica* is absent: The genital atrium opens on the ventral surface a little to the right of the median body line and at about the level of the middle of the anterior testes; it receives the openings of the cirrus sac and metraterm on its posterior and anterior faces respectively (Fig. 5).

The ovary is situated on the right side between the testes; in ventral view it is slightly crescentic in shape, but is somewhat spherical in dorso-ventral section. It is about 0·32 mm. long, 0·28 mm. thick and 0·25 mm. broad (Figs. 1 and 6A). The oviduct is short and is lined with columnar cells bent away from the ovary; antero-dorsally it joins with the Laurer's Canal, which is relatively very muscular and long and may become enlarged just before entering the oviduct; the external opening of the Laurer's Canal can very easily be seen in the dorsal midline in the living specimen when examined under moderate magnification. The yolk reservoir opens into the oviduct on its posterior face soon after it has joined Laurer's Canal; it is a triangular organ, the two points of its base being continued into the transverse vitelline ducts. The oviduct now continues as the ootype and further as the uteroduct which performs several convolutions on itself between the testes and ovary to eventually pass forwards on the left side of the anterior testes and after being coiled once on itself it enters into the uterine sac on its

dorsal surface about 0·8 mm. anterior of the anterior testes. The shell gland is very poorly developed and consists of relatively few cells. The uterine sac extends from the anterior testis to about 0·4 mm. posterior of the acetabulum and contains numerous eggs. The metraterm is a straight canal passing backwards and ventralwards from the right posterior corner of the uterine sac to open on the anterior face of the genital cirrus. The distal portion of the yolk reservoir duct, the ootype and the first portion of the uteroduct are lined by cilia which perform a lashing movement towards the uteroduct.

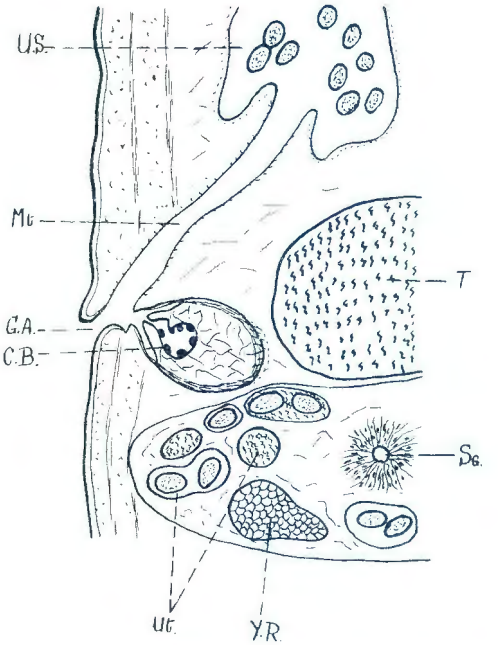

Fig. 5.—Longitudinal section through genital atrium (G.A.). C.B.=cuticular bosses on cirrus. Mt=metraterm. Sg=shell gland. Ut.=sections of uteroduct. U.S.=uterine sac. Y.R.=yolk reservoir.

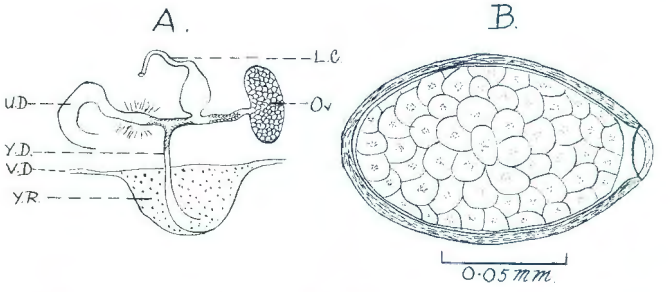

Fig. 6.—A. Reconstruction of proximal portion of female genitalia. L.C.=Laurer's canal; Ov.=ovary; U.D.=uteroduct; V.D.=vitelline duct; Y.D.=yolk duct; Y.R.=yolk reservoir.

B. Egg from mouth of heron.

The eggs (Fig. 6B) are oval, operculated with a relatively thick brown shell; when laid their contents are still in the morula stage. They vary in length from 0·115 to 0·122 mm. by 0·07 to 0·073 mm. broad. The vitellaria consists of numerous isolated glandular follicles extending from behind the acetabulum to the posterior end in two broad lateral bands along the sides of the body except that they meet ventrally under the uterine sac and posterior of the genitalia; two transverse ducts, one on each side, pass inwards and unite with the corners of the yolk reservoir.

Discussion.

From the above description it will be noted that the morphology is very similar to that of *C. lophophallum* Baer, 1933; it, however, differs from this species in that a pharynx is absent and the intestinal caeca open to the exterior through the excretory pore. These two characters together are of sufficient importance to separate this species from all the known species of this genus, and the writer has great pleasure in naming it after the donor through whose interest and kind services the material was made available for study.

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Since writing the above, the writer has received a personal communication from Dr. J. G. Baer, wherein he informs the writer that he has re-examined his sections of *C. lophophallum* and has failed to observe a communication between the intestine and excretory vesicle. He has, however, found some red blood corpuscles in some sections of the excretory canals, and therefore suggests that some communication must exist. A re-examination of the writer's section has failed to reveal any red blood corpuscles in the excretory canals.