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On a Freshwater Annelid of the Genus Bothrioneuron obtained during the "Skeat Expedition" to the Malay Peninsula. By FRANK E. BEDDARD, M.A., F.R.S.

(Text-figures 8-10.)

There are at present only two species of this peculiar genus of Tubificidæ known: they come from such widely separated localities as the neighbourhood of Prague¹ and the neighbourhood of Buenos Ayres². I believe that the facts ascertained from an examination of specimens from the Malay Peninsula justify me in the creation of a third species.

The worms are of about the same size as average specimens of *Tubifex rivulorum*, and thus present no divergence from the two other species of *Bothrioneurow*.

The prostomium is conspicuous and of the ordinary form that it exhibits among the Tubificidæ, as will be seen from an inspection of the two drawings (text-fig. 8, A, B, p. 82).

Prostomial sense-organs.—I find in the present species the same prostomial sense-organ which I described and figured (in section) in Bothrioneuron americanum. It is, moreover, also unpaired in the present species. The position of the organ, however, varies : it is usually on one side, which is preferably the left, just at the junction of the convex upper surface of the prostomium with the lower surface. In one specimen, however, it is exactly in the middle of the lower surface, and in another it is as distinctly upon the upper surface, and also fairly median in position. The organ is very decidedly upon the prostomium itself; it is not situated at the junction of the prostomium with the peristomial segment, as is the case with the corresponding organ of B. americanum.

The one specimen in which the sense-organ happened to be ventral in position is shown in the accompanying drawing, by

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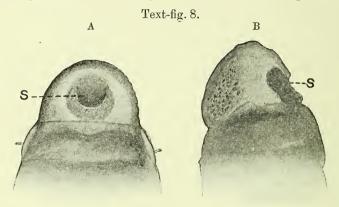
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¹ Stole, "Mon. Ceskych. Tubificid.," Abh. Ges. Böhm. (2) vii. p. 43.

² Beddard, Hamburg. Magalh. Sammelreise, Naiden &c. p. 6.

which it will be seen that a semicircular depression with the concavity directed forwards is the external manifestation of the organ, of which the mass of cells lie behind.

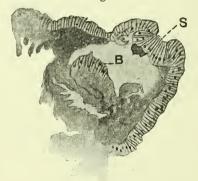
In a specimen in which the prostomial organ is lateral in position (text-fig. 8, B), an external depression is to be seen with equal clear-



Bothrioneuron iris.

Fig. A. Prostomium from below. Fig. B. Ditto from above. S, sense-organ symmetrically placed in A, asymmetrically in B.

Text-fig. 9.



Bothrioneuron iris. Longitudinal section through the prostomium. S, sense-organ; B, supra-œsophageal ganglion.

ness, but naturally in profile. In a longitudinal section (text-fig. 9) of a specimen in which the sense-organ happens to be median and quite anterior in position, the following appearances are observable: the supposed sense-organ consists of a mass of cells which are rather deeper than are those of the surrounding epidermis; this is especially

to be seen at the periphery of the organ, the central part corresponding to the external depression consisting of rather less elongated cells. The considerable size of the organ as compared with the entire prostomium is apparent from the figure and is rather remarkable. It is probable, therefore, that a corresponding organ in other Tubificidæ has not been overlooked.

The setce are, as in the other species, all uncinate and without further complications; there are no subsidiary hooklets between the two prongs in which the free extremity of the seta ends. There appear to be not more than four setæ to a bundle, and very often there are only two.

In this species, as in other Oligochæta, there would appear to be no setæ upon the first segment of the body. However, in longitudinal sections I was able to observe a small mass of muscles upon the first segment of the body, entirely similar in appearance to those which upon ensuing segments enwrap the setæ, and corresponding exactly in position. The mass of muscular fibres was small, but I regard it as a vestige of the seta-bundles of that segment. Setæ are certainly absent ventrally in the neighbourhood of the male pore—a feature in which the present species agrees with *B. americanum*, and differs from the European *B. vejdovskytnum*, in which there are specially modified genital setæ of peculiar form replacing the ventral bundles. The lateral setæ, however, corresponding to the missing ventral setæ are present.

Clitellum.—Bothrioneuron iris differs from its allies in the position of the clitellum, which is pushed back a segment and occupies segments xii. and xiii. In the middle of segment xii. lies the

Male generative pore.—This aperture, single and median, is also a segment farther back than it is in *B. vejdovskyanum* and *B. americanum*. It is interesting to note that there is an apparent connection between the male pore and the clitellum in that an alteration in the position of one is accompanied by an alteration in the position of the other.

The oviducal pores I have not seen.

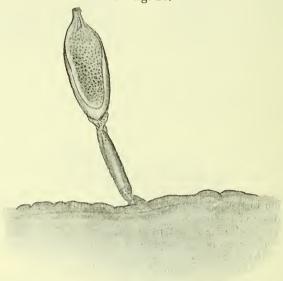
Spermathecal pores are not present. The absence of spermathecæ is one of the characters of the genus.

Spermatophores.—Dr. Stole figures in *B. vejdovskyanum* a crowd of spermatophores attached to the body-wall round the male generative pore. In *B. americanum* I did not find these structures, though perhaps I was a little premature ¹ in using their absence as a specific character. In three out of six mature examples of *B. iris*, I find a single spermatophore apiece also attached close to the male pore. The structure of these spermatophores is similar to those of *B. vejdovskyanum*. There is a thick stalk by which they are attached to the body-wall, which is of a yellowish colour. This stalk is merely attached to the epidermis superficially : it does not penetrate between the cells. Nor can I find any evidence of its

¹ Monogr. Oligocheta, Oxford, 1895. The comparative rarity of the occurrence of spermatophores in *B. iris* may explain the failure to find them in *B. americanum.* They are probably distinctive of the genus. 6*

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being hollow; so far at any rate "hypodermic impregnation" is improbable. The accompanying drawing (text-fig. 10) illustrates the form of the spermatophore, whose structure I have also investigated by transverse sections. Above the stalk it swells out into an oval cup, which is, roughly, of about the same length as the stalk. This narrows rapidly to form a short tube, which appears to be open at the free end. The thickness of the walls of this—the sperm-holding part of the spermatophore—are much thicker below and diminish gradually towards the free tubular extremity of the structure. This part of the spermatophore, as shown in the drawing, does not look as if continuous with the stalk; a slight prolongation of the latter seems to embrace it. The deeply staining contents of the cup appear to be spermatozoa, but their condition of preservation is not sufficiently good to show histological details.



Text-fig. 10.

Spermatophore of Bothrioneuron iris in situ.

Integumental vascular network.—A striking feature of the other two species of this genus is the existence of an integumental network of blood-capillaries. This was so easily to be seen in the examples of *Bothrioneuron americanum* which I examined, that I have some confidence in stating that a vascular integument is not to be found in *Bothrioneuron iris*. I have examined ten or a dozen specimens in glycerine with and without treatment by potash, and I can find no trace of blood-capillaries in the skin. It occurred to me of course that the posterior end of

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the body might be vascular if the anterior end was not, since the tail in the Tubificidæ seems to be often used for respiratory purposes. But here, as elsewhere, I could find no evidence of blood-capillaries in the skin. A specific difference in a feature of such apparent importance is somewhat unexpected. But, as has been, and as will be, seen, the present species is in many ways divergent from its congeners.

Male organs of generation.—As will have been gathered from the account of the external features of the present species, the segments occupied by the various parts of the male generative system are a segment behind those which are occupied by the corresponding organs in the other two species of the genus. In Bothrioneuron iris the testes are in segment xi. instead of x. Excepting in their position, there is nothing especially noteworthy about these gonads. The male efferent apparatus, as in other species of the genus, is complicated and specialised into a number of regions. In transverse sections of the body the ventral surface was seen to be flattened, and thus to contrast with the semicircular dorsal region. At the sides of the body, the flattened under surface was limited by a slightly projecting ridge, so that the outline of a section was somewhat that of a round hat with a brim also in section. In the middle of this area opens the single pore.

When a specimen of the worm is examined in its entirety, the actual orifice is seen to be small and accurately median. In longitudinal sections the smallness of the orifice is also striking. But in transverse sections it appears to be larger owing to the fact that the incurving sides of the body-wall diverge from each other considerably laterally in their course.

The relative size of the male pore would seem to be much that of B. vejdovskyanum as figured by Stolc. But this author does not figure microscopical sections of his species. A noteworthy difference between the two species, which has already been referred to in dealing with external characters, is the total absence of genital setæ in B. iris. In this it agrees with its nearest ally B. americanum. It is unlikely that I should have overlooked these set in two species which have been both of them examined in sections as well as in their entirety mounted in glycerine. There are, in fact, no setæ in the immediate vicinity of the male pore. The terminal male apparatus of B. iris is divisible into the same regions as those which are to be found in B. vejdovskyanum; but their relative dimensions are decidedly different, and there seem to be also differences in their histological structure. The sperm-duct is divided into two different sections as in B. vejdovskyanum. The proximal part, that which immediately arises from the funnel, is about as long as the region which follows, and is much coiled in the middle of a mass of cells which represents a thickened peritoneal investment. This proximal section is of less calibre, and its cells are equally stained by the borax-carmine used in the preparation of the sections; the tube is also of less calibre. The remaining part of the vas deferens also coils about in the midst of the cells mentioned: but

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it is of larger calibre, and the cells are not so thoroughly stained by the carmine; it is, in fact, of a more glandular appearance. This section of tube widens a little before it becomes continuous with the first part of the spermiducal gland proper. The latter is quite different again from the terminal part of the spermiducal gland, a fact which appears to distinguish B. iris from B. vejdovskyanum. In the latter there is, judging from Stolc's figure, no difference whatever between the proximal and the distal regions of the spermiducal gland. In the species with which I am here concerned, the proximal section of the glandular tube is of rather limited extent. Its lumen is fairly wide and is lined by tall columnar cells, which are crowded with rounded spherules of secretion. They are not much stained. In Stolc's figure the "paratrium" is represented as arising from the terminal male tube at about halfway between its commencement and the external orifice. In B. iris the same is the case, and the point of origin nearly represents the passing of the purely glandular part of the spermiducal gland into the distal region, which has quite other characters. The distal part of the tube is wide and has collapsible walls, a fact which is due to their thinness. The epithelium lining the tube here is quite nonglandular. It is a low columnar epithelium which is covered externally by a rather lax covering of muscles.

The paratrium has the egg-shaped form which it exhibits in the other species of the genus. There is, however, no cap of divergent "prostatic" cells such as are figured in B. vejdovskyanum. The paratrium has very thick muscular walls, and its lining of cells becomes deeper and more glandular in appearance at the blind end of the sac.

Where the spermiducal glands open on to the exterior of the body they naturally have to burrow beneath the nerve-cord. The latter is raised to near the intestine, and a slip of muscles forming a thick septum connects it with the ventral body-wall after the two spermiducal glands are separated from each other.

The ovaries lie in the xiith segment; but I have not discovered the oviducts.

The genus Bothrioneuron may be defined and its species discriminated as follows :---

Genus BOTHRIONEURON, Stolc.

Tubificids of 40-50 mm. in length. Setae entirely uncinate. Prostomium with an unpaired, often asymmetrical sense-organ¹. Male pore single and median or paired; atrium with a lateral diverticulum, the paratrium. Spermathecæ absent. Oviducal pores paired². Spermatophores of peculiar form attached to neighbourhood of male orifice.

Hab. Europe; S. America; Malay Peninsula.

¹ Fide Michaelsen, "Oligochæten" in 'Tierreich,' Berlin, 1890, for statement that the sense-organ characterizes the genus. I am unable to read Stolc's paper, which is in Bohemian. ² In *B. vejdovskyanum*. They are not known in the other species.

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1. BOTHRIONEURON VEJDOVSKYANUM Stolc.

B. vejdovskyanum, Stolc, S.B. Böhm. Ges. 1885, p. 647; id. Abh. Böhm. Ges. (2) vii. p. 43; Beddard, Monogr. Olig. 1895, p. 269.

Bothrioneurum vejdovskyanum, Michaelsen, "Öligochæten" in Tierre ch, 1890, p. 54.

Body covered with papillæ. Male pores single and median in xi. Clitellum xi., xii. An integumental vascular network present. Genital seta present on xi. Spermatophores numerous, attached to body-wall in neighbourhood of male pore.

2. BOTHRIONEURON AMERICANUM Beddard.

B. americanum, Beddard, Ann. Nat. Hist. (6) xiii. p. 206; Hamb. Magalh. Sammelreise, Naiden &c., 1896, p. 6; Monogr. Olig. 1895, p. 269.

Bothrioneurum americanum, Michaelsen, "Oligochæten," Tierreich, 1900, p. 54.

Male pores paired on xi. Clitellum xi., xii. An integumental vascular network present. No genital setæ. Spermatophores absent (?).

3. BOTHRIONEURON IRIS, n. sp.

Male pore single and median on xii. Clitellum xii., xiii. No integumental vascular system. No genital setæ. Spermatophores only present to the number of one.