AQUATIC OLIGOCHAETA OF THE INLE LAKE.

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Of the small but interesting collection of Oligochaeta made by Dr. Annandale at the Inlé Lake in the Southern Shan States, and kindly handed over by him to me for examination, the most remarkable specimens are a series of *Branchiura sowerbyi*. These have enabled me to demonstrate the existence of a penis (perhaps a pseudopenis, according to Michaelsen's definition, 5), and to show that in this form also, as well as in *Kawamuria japonica* (11), the muscular coelomic chamber is the apparatus for its extrusion. The amount of variation shown by these specimens in such features as length of body and length and number of gills is surprising.

I have taken this opportunity of also referring to two varieties of *Nais communis* from the Punjab which have recently come into my hands, though these are not part of the Inlé collection.

Family NAIDIDAE.

Genus **CHAETOGASTER.**

Chaetogaster annandalei, Stephenson.

Inlé Lake, S. Shan States. In Sponge (*Ephydatia fluviatilis*) ; 28th February, 1917. N. Annandale. Several specimens, none sexually mature. (No. W. 113-1.)

The identification rests on a comparison with individuals of the original batch of specimens from L. Biwa in Japan (11). The present specimens are about one-fourth larger ; in length a chain of two individuals is ·89 mm., the first being ·63 and the posterior ·26 mm. ; the diameter is ·175 mm. ; the setae of segment ii are 90μ and of more posterior segments 60μ in length.

Chaetogaster limnaei ? V. Baer.

In same tube as the above ; several specimens, none sexually mature. (No. W. 135-1.)

The length of these specimens is no greater, in the preserved condition, than that of *C. annandalei*, with which they occur ; but the other proportions are quite different ;—they are about twice as thick, and the setae also are markedly larger. So far as I can see, the only distinction between these specimens and *C. limnaei* is the rather smaller number of setae in these,—6 or 7 in segment ii and 3, 4, or 5 behind. *C. limnaei* has up to 8—12, according to Vejdovsky (12), who also remarks that the hinder segments have commonly more than the anterior ; this is not the case here, but it is pretty certain that the above numbers do not represent the actual state in the living animal, and that a number of setae

have fallen out ; in some segments I cannot discover any setae, and in one bundle I saw only one, though succeeding segments had four or five. The identification is however by no means above doubt.

C. limnaei is a European species which is parasitic on and in fresh-water Gastropods, or is occasionally free-living. It has not hitherto, I think, been recorded as living in sponges.

Chaetogaster bengalensis, Annandale.

Small canal and flooded rice-fields near Than-taung on W. Side of Inlé Lake, Southern Shan States ; in *Ephydatia fluviatilis*. 28th February, 1917. N. Annandale. A number of specimens, none sexually mature. (No. W. 114-1.)

The identification rests on a comparison with the types of the species, kindly sent to me by Dr. Annandale. The species was originally obtained by him from water snails in Calcutta (1). I may supplement the original account by a few additional particulars, based on an examination of the present specimens.

The length of a chain of two individuals in the preserved condition is 1·8 mm. or more ; of the first individual of a chain measured separately, 1 or 1·2 mm. The diameter at the widest part is ·35—·38 mm. The comparatively short length just given does not conflict with Annandale's statement that the animal measures at least 10 mm. when fully expanded ; the type specimens are the same length as these.

$N=10$ or 11.

The setae of segment ii are in length 85μ, 90μ, and 104μ in three different specimens. The main portion of the shaft is straight, the prongs are almost equal in length and thickness as a rule, even to the oil immersion lens ; sometimes the proximal prong appears slightly thicker at the base. The position of the nodulus varies from the middle of the shaft to frankly distal (distal to nodulus : proximal to nodulus : : 2 : 3).

In more posterior segments the setae are shorter, 68μ—74μ in length ; in thickness they are about 1·7μ. Here again the greater part of the shaft is straight, the distal end being hooked, and the proximal gently curved ; no difference can be regularly made out between the terminal prongs, though sometimes the distal seems to be rather longer and thinner. The nodulus varies in position in the setae of the same bundle,—from the middle of the shaft to distinctly distal ; where the disposition could be minutely examined, the innermost seta of the bundle has the nodulus nearest the middle, and the most external seta has it most distally placed on the shaft (*cf.* Stephenson, 9*a*).

The number of setae in a bundle is, as Annandale has remarked, very large ; I counted 16 (as well as lesser numbers) both in the bundles of segment ii and in those further back (Annandale, 15—17). The much-curved line of insertion of the setae of a bundle is very striking.

The prostomium is practically absent,—it is merely the anterior lip of the mouth ; this is a large circular orifice, ventro-terminal, looking obliquely forwards and downwards. The section of the alimentary canal which succeeds the pharynx, usually called oesophagus, is short but quite distinct. The beginning of the next part of the canal, the swollen crop, is marked by a number of cells, arranged in a fairly broad ring

around the opening of the oesophagus into the crop ; these are part of the lining epithelium, as is seen in longitudinal sections, where they appear as prominent cells projecting into the lumen, almost constituting a circular valve.

There is a considerable granular more opaque mass in the cerebral ganglion, as in some other species of the genus (*cf.* Stephenson, 6).

Remarks.—The species is a well marked one, the large number of setae being very characteristic. In addition, the practical equality in length and thickness of the terminal prongs of the setae, even to the highest powers (correctly shown in Annandale's figure), with the short but distinct oesophagus, will also serve as good marks of distinction.

Not having noticed, in those species of *Chaetogaster* which occur in the Punjab, any specially curved line of insertion of the setae, I was much struck by this very marked feature in the present specimens ; the curvature seemed to me to be even more accentuated than in Annandale's figure. It is not, however, peculiar to this species ; Miss Davies, in describing *C. australis* (3), which has resemblances to the present species, mentions that the setae are arranged in the form of a semicircle, except in the case of those of segment ii ; Mdlle. Dehorne mentions it in her study of *C. diaphanus* (4), and adds that this arrangement is even more distinct in *C. limnaei.*

I do not add to the list of distinctive features of *C. bengalensis* the presence of a posterior sucker (the anterior sucker of Annandale is the margin of the mouth, as in the case of the leech). The posterior sucker is mentioned by Annandale in his original account ; in *C. australis*, Miss Davies says, " at the posterior end there is no definite sucker, but the animal seems capable of slightly flattening its body so as to somewhat resemble one " ; and for *C. victoriensis*, " movement takes place by means of a series of contractions and expansions with the aid of anterior and posterior suckers, somewhat like a leech." I have not been able to see the posterior sucker in the types of *C. bengalensis*, nor in the present batch of specimens ; in Annandale's figure it appears to be merely the margin of the anus,—but this aperture is not provided with any special musculature discoverable either in the examination of mounted specimens or in longitudinal sections. Notwithstanding the more or less definite statements I have quoted above, I do not think there will be found in any species of *Chaetogaster* a posterior sucker, that is, a definite muscular organ, whether including the anus or not. I believe that the attachment of the animal at the posterior end takes place by means of the hinder setal bundles, the hooked ends being turned forwards and taking hold of the substratum (as in the case of backward progression, *cf.* Stephenson, 6, p. 237) and that Mdlle. Dehorne (on *C. diaphanus*) correctly likens the mode of progression to that of a caterpillar,– " l'animal se déplace à la façon des chenilles arpenteuses, les soies bucco-pharyngiennes jouant le rôle de harpons, les soies moyennes et postérieures fixant la chaîne au substratum."

If I might venture an additional word of criticism, it is that sections do not show any special thickness of the pharyngeal wall ; nor is there any peculiarity, as Annandale supposes, in the manner of insertion of the setae of segment ii.

Genus **NAIS.**

Nais communis, Piguet var. **punjabensis,** Stephenson.

Kasauli ; July, 1915. Baini Prashad. Several specimens, none sexually mature.

Nais communis, Piguet var. **caeca**, Stephenson.

Along with the above. Several specimens, none sexually mature.

It is interesting to find the blind variety of this worm along with the one possessing eyespots, as was the case in the material from Travancore from which the var. *caeca* was first described (Stephenson, 7).

Family TUBIFICIDAE.

Genus **BRANCHIURA.**

Branchiura sowerbyi, Bedd.

Inlé Lake, Southern Shan States. In very soft mud in the open lake in 7 feet of water ; green plants abundant. Several batches, 19th February to 5th March, 1917. N. Annandale. (No. W. 103-4—1.)

Kaung-daing, Yawnghwe State, Southern Shan States. In soft mud at edge of stream of warm water issuing from hot sulphur spring ; surface of mud barely covered with water ; no vegetation. Several specimens ; February, 1917. N. Annandale. (No. W. 111—1.)

Inlé Lake, Southern Shan States. In black mud at edge of lake in about 1 foot of water ; much decaying vegetation present. Several specimens ; 28th February, 1917. N. Annandale. (No. W. 112—1.)

Of the specimens collected at the various stations a number were sexually mature ; and a preliminary examination showed in some a feature not hitherto recorded,—a penis-like projection from the male orifice. This was however not present in all the sexual animals ; one showed a projecting penis on one side and not on the other ; a few showed two, the majority no penis at all. Three specimens were sectioned,—with none, one, and two penial projections respectively.

In those cases where there is no projecting penis the various structures have much the arrangement described by Michaelsen (5). Michaelsen divides the male deferent apparatus into the following parts :—

(*i*) the funnel ;

(*ii*) the vas deferens, which enters the wall of the next portion, the atrium, near the apex of the latter, and runs in that wall, in a direction away from the external aperture, to its very tip ;

(*iii*) the ental [1] portion of the atrium, a moderately wide tube surrounded by a thick layer of modified peritoneal cells ;

(*iv*) the middle portion of the atrium, a narrow continuation of the above, which soon enters the coelomic sac, in the upper part of which it winds about, accompanied by the paratrium ; the lumina of the atrium and paratrium finally unite ;

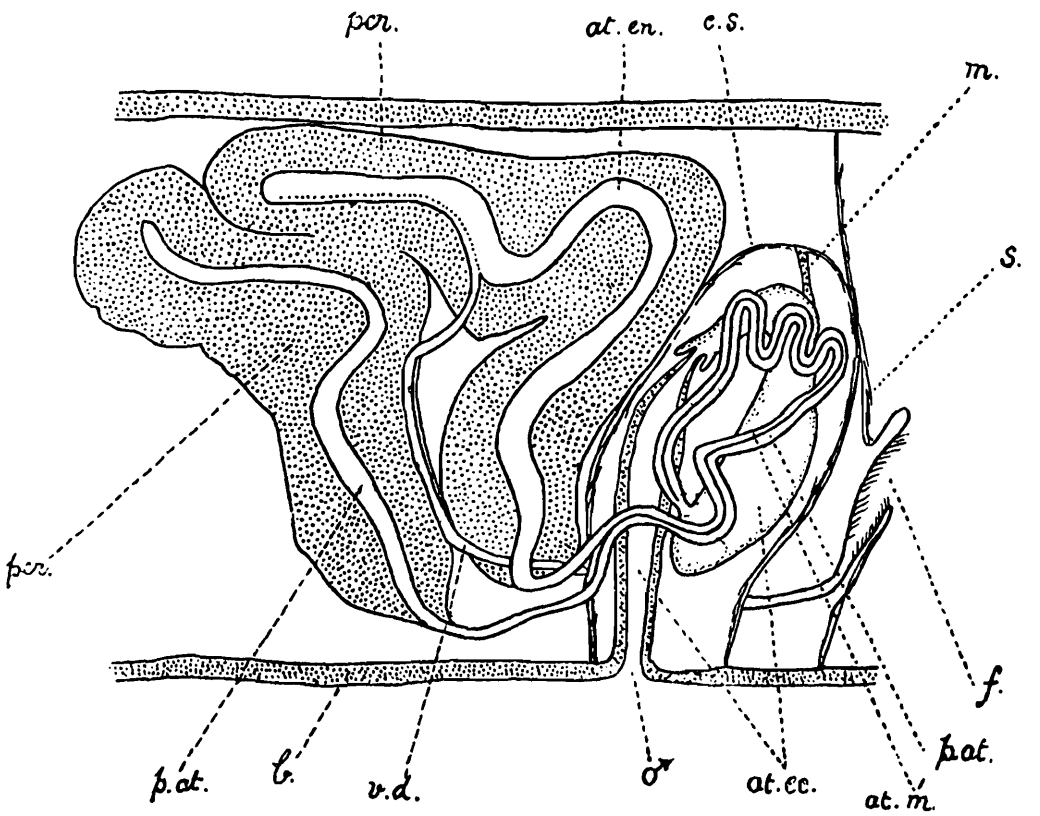
[1] Different authors use the terms " proximal " and " distal " in different senses in describing, for example, such a structure as the atrium, or a spermatheca. The more usual practice amongst English writers seems to be to take the fixed end,—that which is united with the bodywall,—as the proximal ; but Michaelsen calls the internal end proximal and the outer distal. To obviate confusion I use the terms " ental " and " ectal."

(*v*) the wider, ectal portion of the atrium has a generally vertical course to the exterior through the coelomic sac ;

(*vi*) the paratrium, a narrow tube ending blindly at its ental end and joining the atrium at the other ; it is also covered by a thick layer of modified peritoneal cells.

In all the cases hitherto observed the male orifice was quite simple (Beddard, 2 ; Michaelsen, 5 ; Stephenson, 9).

I may observe, first, that the ectal portion of the atrium is here divisible into two distinct sections,—a lower, the terminal part of the whole deferent apparatus, and an upper ; the distinction is, I believe, of some importance from the point of view of the protrusion of the penis. The upper section makes several bends in the upper part of the coelomic sac ; the epithelium lining the lumen is cubical, and the peculiarity of the cells is that the inner portion stains slightly or not at all,—less deeply than the basal part ; the hyaline appearance of the part of the cells which is towards the lumen gives them a distinctive character ; the nuclei are spherical, and stain rather lightly, showing scattered grains of chromatin in their interior. This section is divided from the lower usually by a distinct narrowing, and sometimes the walls of the tube appear folded here. The lower section has a generally vertical position in the sac ; its muscular coat is thick, and the epithelium is columnar, though of irregular height ; the nuclei are oval, and stain densely.



1.—*Branchiura sowerbyi ;* male genital apparatus, diagrammatic. *at. ec.*, *at. en.*, *at. m.*, the ectal, ental, and middle portions of the atrium ; *b.*, bodywall ; *c.s.*, coelomic sac ; *f.*, funnel ; *m.*, muscular band ; *p. at.*, paratrium ; *per.*, mass of peritoneal cells ; *s.*, septum 10/11 ; *v. d.*, vas deferens ; ♂ male aperture.

There are also a few other minor differences between these specimens and those investigated by Michaelsen. Thus the middle does not

suddenly become swollen where it passes into the ectal portion of the atrium,—the enlargement is gradual. The atrium extends as far as, and may extend further back in segment xii than the paratrium ; both atrium and paratrium may be confined to segment xi. The portion of the vas deferens which is contained within the atrial wall is considerably greater than is shown in either of Michaelsen's figures (see the dotted line in fig. 1), and indeed the length of the ental portion of the atrium as a whole is here much greater, and its course more winding ; it is here a much more conspicuous feature of the anatomy than the paratrium, though the reverse would seem, from the figures, to have been the case in Michaelsen's specimens. The lower (ectal) portion of the paratrium, of considerable length, has here a well-marked lumen and is lined by cubical cells ; it has here escaped from the voluminous peritoneal investment. Michaelsen seems to be right in denying a muscular coat to the paratrium, at any rate to that part which is enclosed in the thick covering of peritoneal cells. Fig. 1 gives a diagrammatic representation of the male apparatus in the present specimens.

The penis, where it occurs, appears as a pear-shaped or cylindrical projection, sometimes twisted, from the male orifice. It is an evagination of the ectal portion of the atrium ; and it is here that the distinction of the ectal portion into two sections, an upper and a lower, is of use ; the lower part forms, when protruded, the outer wall of the penis, and the upper the axial canal which traverses the projection (text-figs. 2 and 3) ; the aperture of the protruded penis is thus the junction

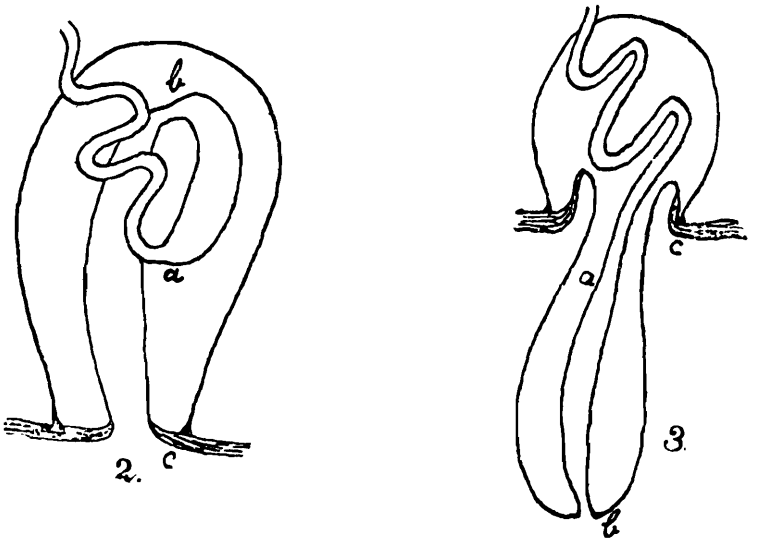


FIG. 2.—*Branchiura sowerbyi* ; penis not protruded.
„ 3.— „ „ penis protruded.
a, *b*, *c*, corresponding points.

between the upper and lower sections. This is borne out by the characters of the epithelium ; the central tube is lined by cells of a cubical shape with a more lightly staining inner portion ; but it is not so easy to recognize in the outer covering of the penial projection the characters of the cells lining the lower part of the atrium, since these are for the most part much flattened in their new situation.

When the penis is protruded the coelomic sac extends to only about half the height of the segment, and contains only the winding portions

of the conjoined atrium and paratrium ; the evagination of the terminal section of the atrium seems never to be complete, so that there is in full protrusion still a deep groove round the base of the penis, which thus projects from within the male aperture (text-fig. 3).

I was at one time inclined to doubt whether the " coelomic sac " was really coelomic,—whether its cavity was really a cut-off portion of the coelom. It seemed to me that the most terminal portion of the male duct,—that part which was originally included in the parietes,—might have hypertrophied to produce the penis, and in so doing might have raised up the inner part of the muscular layer of the bodywall so as to form the sac. On this supposition the cavity of the sac would not be coelomic, but only an enlarged split in the muscular wall of the body. In the same way the cavity of the gills is a space, not coelomic in origin, between the two muscular layers of the parietes ; there the outer muscular layer carrying the superficial epithelium projects outwards as a gill, here the inner layer carrying the peritoneal investment would project inwards as the sac-wall.

However, the atrium within the sac has an (apparently) peritoneal covering, well-marked in places, and consisting of cubical clear cells ; and a much flattened cell-layer can also be seen on the inner side of the sac-wall. The cavity and its contents appear therefore to be lined and covered respectively by peritoneal epithelium, and the space to be really coelomic.

The above condition is remarkably similar to that which I have recently described in *Kawamuria* (11). It is curious that the penial projection in *B. sowerbyi* has not previously been observed ; there is however nothing, in the more usual condition of the orifice, to indicate the possibility of such a protrusion,—the canal ends quite simply on the surface of the body. It is true that I recognized the function of the coelomic sac in *Kawamuria*,—to cause, by contraction of its walls, the extroversion of the contained tube ; but in *Branchiura*, which has the sac but, so far as I then knew, no protrusible penis, I considered the sac to have lost its function, and to be a rudimentary organ ; it is evidently at times fully functional.

The distance between *Branchiura* and *Kawamuria* is thus reduced ; the separation must now depend on the presence or absence of gills. I will not further discuss at present whether generic distinction is still justifiable, but may refer to what I wrote in my former paper.

The specimens showed much variation. In length one batch consisted of worms of 30, 28, 25 or fewer mm. ; in another the individuals were 45—70 mm. ; in others they were 90 or 100 mm., and one specimen reached the great length of 185 mm.

The number of pairs of gills was 40—47 in the shorter worms, and 90 in the longest ; but this was not the maximum. In one fragment 140 pairs were found, but the total length of the animal cannot be known. As many as 110 were found in an individual only 70 mm. long.

Sometimes nearly all the gills were well developed, only a few at the anterior end of the series being represented by mere tubercles ; in others a large number of the anterior gills were only tiny projections. But variation in this point seems to have no relation to the number of

gills or the size of the animal ; in a posterior fragment with 118 pairs, 43 were mere tubercles, and indeed all but the last 20 were little more ; in a specimen 100 mm. long, with 92 pairs, all but a few were well developed ; in the longest specimen, with 90 pairs, about half were tubercles only.

The length of the gills also varies. In an ordinary specimen they are perhaps somewhat shorter than the diameter of the body ; but in two examples of the present series they were very long,—about three times as long as usual,—filamentous, tangled together and hence difficult to count. In a third specimen of the same batch they were about twice as long as usual ; but in the fourth and last in the tube they were not noticeably longer than the ordinary.

As can be seen, these variations seem to be independent of each other ; nor can I connect them with the habitat except in a small degree. The specimens just mentioned, with the very long gills, were all taken from black mud at the edge of the lake, in about one foot of water, where much decaying vegetation was present ; the length might be correlated with deficiency of oxygen,—but one specimen had gills of only normal length. The length of the animals may however have a relation to the nature of the bottom in which they live ; thus those living in soft mud at the edge of a stream were the shortest (the stream was warm, and issued from a hot sulphur spring,—conditions which might perhaps have checked growth) ; lengths of 45—70 mm. were found in one foot of water in relatively stiff, peaty mud ; and specimens 90, 100, and 185 mm. were contained in the catches from the open lake, in seven feet of water. These very long specimens were taken on a bottom of extreme softness, indeed of semi-liquid consistency, in which it would be necessary for cylindrical bodies to be of great length in order to maintain a vertical position.

Family MEGASCOLECIDAE.

Perionyx fulvus, Stephenson.

Inlé, Yawnghwe State, Southern Shan States ; soft mud in muddy stream in 1½—3 feet of water. 6th March, 1917. N. Annandale. Four specimens. (No. W. 108-1.)

The species was hitherto only known from a single specimen taken in Calcutta. I must here correct a mistake which has crept into my original account (10) ; the male pores are there said to be " not very close together on segment xviii." I do not know how the word " not " crept in ; my original notes have " male apertures very close together," and the figure of the male area in my notes (which I did not reproduce in the paper) is practically a facsimile of the one I give here (fig. 4), drawn from the present specimens, except that the apertures are there even rather nearer together.

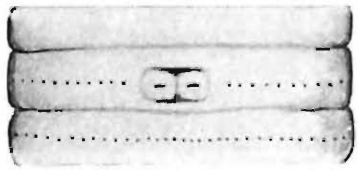


Fig. 4.—*Perionyx fulvus ;* region of male apertures.

As the type specimen is incomplete posteriorly, I may give the following measurements :—length 175 mm., thickness 4 mm. (max. 4·5) in

the case of the largest example ; the others are smaller,—one which is only 98 mm. long and 2·5 to 3·25 mm. in thickness has well marked male apertures though no clitellum. The largest specimen had 178 segments.

The only notable difference of the specimens from the type is the colour ; most species of *Perionyx* are distinguished by a rich purple colour dorsally, and the fact that the type specimen was yellowish brown and almost unpigmented suggested the specific name. The present examples however are a deep brownish purple above, pale below. The aquatic habitat is interesting.

An immature *Perionyx* was also obtained from the Inlé Lake, Southern Shan States, from black mud at the edge of the lake, in about one foot of water where much decaying vegetation was present. The locality, and the fact that it was also aquatic, suggest that the specimen belongs to the same species ; and this is borne out to some extent by the commencing change in the male area, where the transverse grooves before and behind the male apertures, characteristic for *P. fulvus*, are beginning to appear. I mention it because of its colour, which seems to represent an intermediate condition between the fulvous and purple. To the naked eye it appeared a dusky purple dorsally in the anterior part, becoming increasingly lighter behind, and in the posterior half it is merely buff or tawny. Under the binocular dissecting microscope the colour is uniform at the anterior end ; but behind this, longitudinal streaks of pigment are seen in each segment, purple on a yellow background, interrupted by the intersegmental furrows and not always corresponding in position from one segment to the next ; the streaks are still present, but increasingly lighter, up to the hinder end, but there they only suffice to modify the yellow background to a buff tint (fig. 5). The deposition of pigment thus appears to take place in streaks, and not uniformly, a uniform tint being produced by expansion and coalescence of the streaks.

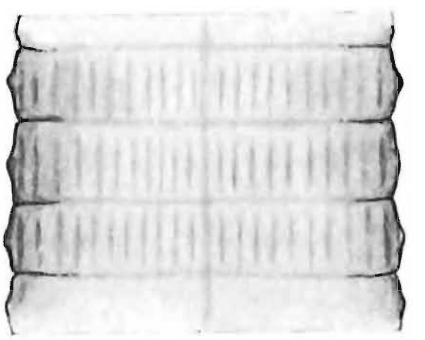


FIG. 5.—*Perionyx fulvus* (presumably) ; to show manner in which pigmentation develops.

REFERENCES TO LITERATURE.

1. ANNANDALE, N.—Notes on an Indian worm of the genus *Chaetogaster*. *Journ. and Proc. As. Soc. Bengal*, n. s. vol. i, 1905.
2. BEDDARD, F. E.—A new Branchiate Oligochaete (*Branchiura sowerbyi*). *Quart. Journ. Mic. Sci.*, n. s. vol. xxxiii, 1892.
3. DAVIES, OLIVE B.—On two new species of *Chaetogaster*. *Proc. Roy. Soc. Victoria*, n. s. vol. xxvi, 1913.
4. DEHORNE, LUCIENNE—Les Naidimorphes et leur reproduction asexuée. *Arch. Zool. exp. et gen.*, t. lvi, 1916.
5. MICHAELSEN, W.—Zur Kenntnis der Tubificiden. *Arch. Naturgeschichte*, lxxiv, Jahrg., 1908.

6. Stephenson, J.—Descriptions of two freshwater Worms from the Punjab. *Rec. Ind. Mus.*, vol. i, 1907.

7. ,, —On some aquatic Oligochaete Worms commensal in *Spongilla carteri*. *Rec. Ind. Mus.*, vol. v, 1910.

8. ,, —On some aquatic Oligochaeta in the collection of the Indian Museum. *Rec. Ind. Mus.*, vol. vi, 1911.

9. ,, —On a new species of *Branchiodrilus* and certain other aquatic Oligochaeta, with remarks on Cephalization in the Naididae. *Rec. Ind. Mus.*, vol. vii, 1912.

9*a*. ,, —On a rule of Proportion observed in the Setae of certain Naididae. *Trans. Roy. Soc. Edin.*, vol. l, 1915.

10. ,, —On a collection of Oligochaeta belonging to the Indian Museum. *Rec. Ind. Mus.*, vol. xii, 1916.

11. ,, —Aquatic Oligochaeta from Japan and China, in. Zoological Results of a Tour in the Far East, ed. N. Annandale. *Mem. As. Soc. Bengal*, vol. vi, 1917.

12. Vejdovsky, F.—System und Morphologie der Oligochaeten. Prag, 1884.