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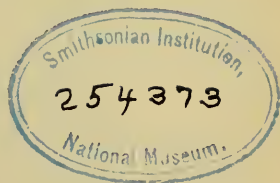
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28. On the Sexual Phase in certain Indian Naididæ (Oligochæta). By HARU RAM MEHRA, M.Sc., Professor of Zoology, Hindu University, Benares*.

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(Text-figures 1-3.)

I have recently collected in the neighbourhood of Agra a large number of examples of the following species of Naididæ and Tubificidæ, many of which are fairly common there :—

Nais pectinata var. *inequalis* Stephenson.

Nais communis Pignet, var. *punjabensis* Stephenson.

Hæmonais laurentii Stephenson.

Chaetogaster orientalis Stephenson.

Chaetogaster punjabensis Stephenson.

Dero limosa Leidy.

Pristina longiseta Ehrbg.

Branchiodrilus hortensis Stephenson.

Branchiura sowerbyi Beddard.

As is well known, the Naididæ usually reproduce asexually by fission, and in many species the genital organs have never yet been described. As Stephenson remarks (3), if such descriptions "were available throughout the group, it can hardly be doubted that we should be able to judge better of the affinities of genera and species, and consequently to improve our classification; since the diagnoses of species and genera, and the scheme of classification, depend at present to an unduly large extent on one single set of characters, the form and distribution of the setæ." I therefore give an account of the sexual organs in two of the above species, *Nais pectinata* var. *inequalis* and *Branchiodrilus hortensis*; though the organs have been described in certain other species of *Nais*, we have as yet no account of them in any species of the genus *Branchiodrilus*.

All the species of Naididæ which have been observed by Stephenson to become sexual in Lahore, considerably further north than Agra, do so from February to May; the rains are there later and scantier than further south, and May, June, and sometimes July, before the rains appear, when the ponds are dry and the ground baked hard, represents the most unfavourable season of the year for pond-life. In Europe these worms would seem usually to enter on the sexual phase in the autumn, before the rigours of winter. In Agra I found the sexual specimens described below in the autumn—in this part of the country the rains are abundant from the latter part of June to September; the ponds begin to dry up in October, and the cold weather

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appears, as in Europe, to be the unfavourable season. Whether the sexual phase makes its appearance in spring or autumn, therefore, it seems to be a measure of protection against approaching adverse conditions; the ova, quiescent or developing slowly within the cocoon, are probably able to withstand such conditions better than the adult animal.

In the case of *Nais pectinata* var. *inequalis*, after the attainment of the full sexual phase, the alimentary canal in several of my specimens was seen to degenerate; the same phenomenon has been noticed by Stephenson (3, 4) in *Dero limosa* and *Hæmonais laurentii*.

Many specimens of *Branchiura sowerbyi*, *Dero limosa*, *Branchiodrilus hortensis*, and *Hæmonais laurentii* were found living together in the mud of a pond near Sikandra. A similar curious association of *Branchiura sowerbyi* and a species of *Branchiodrilus* has been noted by Beddard (1) in the Victoria regia tank in the Royal Botanic Society's Gardens in Regent's Park; and *Branchiura sowerbyi*, *Branchiodrilus hortensis*, and a species of *Dero* have been found associated by Stephenson under natural conditions at Lahore (2). These three are among the few genera of Oligochaeta which possess gills; in *Hæmonais laurentii*, the fourth worm which I found in the association, though there are no gills, the vascular system is, for one of the Naididæ, particularly highly developed.

NAIS PECTINATA var. INÆQUALIS Stephenson.

Since Stephenson had only spirit specimens at his command, I prefix a short account of some features of the general anatomy of this worm.

My specimens were larger than Stephenson's, their usual length being 8-10 mm., but when the worms are fully extended it may reach 15-18 mm. The colour is light reddish brown. The prostomium is bluntly conical. The worms exhibit active wriggling movements. The number of segments varies considerably—from 40 to 95.

The ventral setæ are 4-6 in a bundle, usually 5; the length of those in the anterior segments (ii.-v.) is 97-105 μ , of the rest 88-93 μ . The dorsal hairs were 306-332 μ , and the dorsal needles 106-112 μ ; there is a slight indication of a nodule on the latter.

The penial setæ (text-fig. 1) are the modified ventral setæ of the sixth segment. They are 4-6 in a bundle, 98-105 μ in length, and are somewhat swollen near the tip, which is usually not forked although it is slightly hooked. Only two setæ were noted as being bifid at their free end, and in these the prongs were short, blunt, and of equal length. The whole bundle has somewhat the appearance of a fan, and arises to the inner side of the male genital aperture.

Ordinarily the dorsal setæ begin in the sixth segment, as

usual in the genus. In sexually mature specimens, however, they begin in segment viii.; in one specimen there were needle setæ only in the dorsal bundles of viii., but no hairs; in a few the setæ only began in ix.; in two cases the setæ were seen to be thrown off from segment viii. when a cover-glass was gently placed over the worm,

The body-cavity contains a large number of rounded corpuscles, brownish in colour, and in addition there are a few colourless corpuscles filled with refractile granules. Cœlomic corpuscles were more numerous in specimens which had been kept in the laboratory for several days. They are few in the first six segments.

Text-figure 1.



A. Penial seta of *Nais pectinata* var. *inæqualis*. $\times 540$.

B. Penial seta of *Branchiodrilus hortensis*. $\times 540$.

Text-fig. 1 drawn by camera lucida.

The pharynx occupies segments iii.-v., and is diffusely covered by a small amount of chloragogen pigment, which extends right up to the prostomium. In a transverse section the cavity has the appearance of an inverted T, owing to the presence of a median dorsal diverticulum; this diverticulum and the dorsal wall of the pharynx are ciliated. On the upper and lateral surfaces of the pharynx are a number of pyriform cells, their narrow ends resting on the surface of the pharynx; these are arranged in groups of three, four, or more, and the groups are separated by strands of muscle which pass upwards from the pharynx to the body-wall. The cells are about 30μ in length and 9μ in thickness; they stain deeply with hæmatoxylin. The whole pharynx much resembles that of *Hæmonais laurentii* (4).

The gut is not distinctly differentiated into œsophagus, stomach, and intestine; it is somewhat larger in segments vi.-viii., then narrow as far as xiv., after which it is continued as a fairly broad tube for some distance. Its epithelium is ciliated. The anus; is

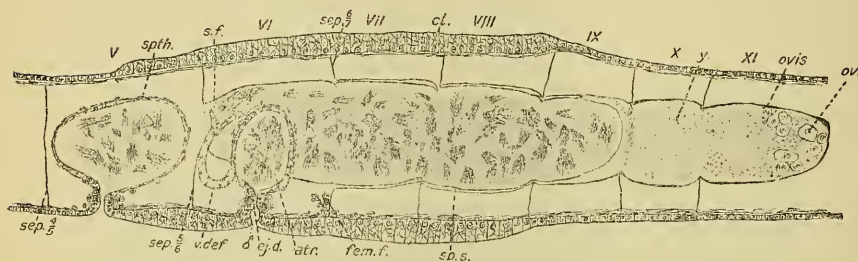
dorsal. Strong ciliary movements were seen in the posterior part of the gut, the direction being forwards; antiperistaltic contractions were also noticed to be taking place over some length of this part of the tube.

The blood is yellowish, and without corpuscles. The dorsal vessel lies on the left side of the alimentary canal near the ventral surface as far as septum 5/6, where it becomes dorsal, and lies over the pharynx; it is surrounded by chloragogen cells, or in the region of the pharynx by the pigment previously mentioned. It bifurcates near the anterior end of the animal, and the branches, turning ventrally, unite to form the ventral vessel at the level of the first ventral setal bundles. There are four pairs of lateral commissures in the pharyngeal region, which form a plexus; behind this, from segment vi. onwards, there is a commissure on the anterior face of each septum,—in some specimens, however, these were only seen as far back as segment xvi. The body-wall is devoid of capillaries.

The first nephridium lies in segment vii.

The cerebral ganglion is large and bilobed, deeply indented in front and behind. The ventral nerve-cord has an irregular lobulated outline, ganglia not being clearly distinguishable.

Text-figure 2.



Genital region of *Nais pectinata* var. *inaequalis*. *Atr.*, atrium; *ej.d.*, ejaculatory duct; *fem.f.*, female funnel; *ov.*, ovum; *ovis.*, ovisac; *sep.* 1/5, 5/6, and 6/7, the septa between segments iv. and v., v. and vi., and vi. and vii.; *s.f.*, seminal funnel; *sp.s.*, sperm-sac; *spth.*, spermatheca; *v.def.*, vas deferens; *y.*, yolk; *♂*, male aperture. \times ca. 120.

Text-figs. 2 and 3 are semidiagrammatic, and are compiled from several successive vertical sections.

Genital Organs (text-fig. 2).—As usual in the Naididae, the gonads appear first, and disappear entirely before the rest of the genital apparatus has reached its full development.

The testes are a pair of ovoid bodies attached to the posterior face of septum 4/5. The ovaries are similar in appearance in the living specimen, but smaller; they arise from the posterior face of septum 5/6.

The seminal vesicle is formed soon after the appearance of the gonads, as a backward bulging of septum 5/6, which later on,

when distended with the male products, may reach as far back as segment xii. The vas deferens, one on each side, is a short tube with only a single bend; in diameter it is 15μ , except where it joins the atrium, where it is only 9μ ; it has a uniform lining of cubical epithelial cells. It passes vertically downwards from the funnel on the posterior face of septum 5/6, and then after a slight bend enters the atrium on its anterior aspect close to the origin of the ejaculatory duct.

The neck of the male funnel lies in the mouth of the sperm-sac (seminal vesicle). The funnels fill up the mouth of the sac, and are directed upwards and backwards just within it; they meet each other in the middle line above the gut by their inner margins, and their outer surfaces are fused with the contiguous part of the wall of the sac. They are cup-shaped, with everted lips, and are lined by columnar ciliated cells with prominent oval nuclei at the base. The greatest diameter of the funnel is about 45μ .

The atria are ovoid chambers with their long axes vertical, lying one on each side of the seminal vesicle in segment vi. Each is $80-90\mu$ in height and $45-50\mu$ in breadth, and is lined by an epithelium of cubical cells with indistinct outlines and conspicuous nuclei; outside the epithelium is a thin coat of circular muscular fibres, outside which again the peritoneum is indicated by a few scattered nuclei. The lumen may contain spermatozoa or only a little coagulum. The ejaculatory duct is short, about 30μ ; its epithelium consists of closely packed columnar cells with large peripherally situated nuclei; it has a fairly thick investment of circular muscular fibres. The duct may be invaginated into the base of the atrial cavity; it opens to the exterior in the depth of a short tubular depression of the ventral body-wall, about 15μ in length, which is narrower at the surface, and broader above, where it receives the duct. There are no "prostatic" cells in connection with vas deferens or atrium.

A thick band of muscle-fibres runs vertically upwards from the ventral body-wall, lying internal to the atrium and supporting the setal sac containing the penial setæ.

The ovisac, formed by the backward bulging of septum 6/7, surrounds the sperm-sac which lies within it. It may reach back to segment xvi.; it contains a large mass of yolk granules, which stain faintly with eosine; and in its hinder part a number of ova. The septa of the several segments behind the seventh retain a transverse position between the body-wall and the ovisac, fusing closely with the periphery of the latter. Large blood-vessels are seen closely applied to the sperm-sac and inner face of the ovisac.

The female funnels are attached to the anterior face of septum 6/7 near the ventral parietes; the cells lining it are small, and appear to be modified peritoneal cells containing little else than nuclei. In one specimen the funnels were seen to open on the ventral surface at about the level of septum 6/7. They are seen

only in specimens which have reached full sexual maturity, and considering the large size of the ova seem to be too minute to be of any functional importance.

The spermathecae occupy segment v., and their openings lie at the anterior edge of the clitellum immediately behind septum 4/5. The ampulla attains a maximum height of 105μ ; its posterior surface lies near the mouth of the sperm-sac, while in front it may push forwards septum 4/5 so as to encroach on segment iv. The ampullae when distended are ovoid, and meet and press on each other in the middle line above the alimentary tube. The epithelium is low and flat, except near the duct where the cells are fairly high and cubical. There is a thin layer of circular muscular fibres, and a few peritoneal cells on the outside. The spermathecal duct arises anteriorly instead of from the middle of the base of the ampulla; it is slightly oblique in position, cylindrical, about 45μ in length; its lining consists of closely packed columnar cells with nuclei peripheral, and a fairly thick coat of circular muscular fibres surrounds this epithelium.

The clitellum covers more than half of segment v., and all vi., vii., and viii.; to the naked eye it is opaque white. The cells are four times as high as the ordinary surface epithelium, are vacuolated, and when fully developed lose their distinctness of outline. The clitellum is absent from the regions of the body-wall between the spermathecal pores and the male apertures.

The alimentary canal undergoes great degeneration in the sexually mature worm. Though known in *Dero limosa* and *Haemonais laurentii*, the phenomenon has not so far been observed in the genus *Nais*. In advanced stages of maturity the mouth becomes closed; the buccal cavity and anterior part of the pharynx lose their lumen and become reduced in size; the pharyngeal cells lose their distinctness of outline, and those of the ventral wall are reduced in size, low and cubical. Behind the pharynx the gut is continued as a narrow band without a lumen as far as segment xii.; the cells lose their regular epithelial arrangement, and the solid cord is, seen in section, smaller than, or sometimes about the same size as, the ventral nerve-cord: there may be small spaces here and there, filled with fluid; there are large blood-vessels around it, in close contact with it. Behind the sperm-sac there are small cavities in the solid cord of disintegrating cells; but after segment xvii. the intestine, though still narrower than in the normal worm, retains its proper form, and is lined with columnar cells surrounded by chloragogen cells; the lumen is either empty or contains some granular matter. Although the gut is thus degenerating in the anterior part of the body, the nephridia are normal, the blood-vessels are larger than usual, and the same is the case with the cerebral ganglion; the specimens manifested the characteristic wriggling movements, and were thus in no way pathological.

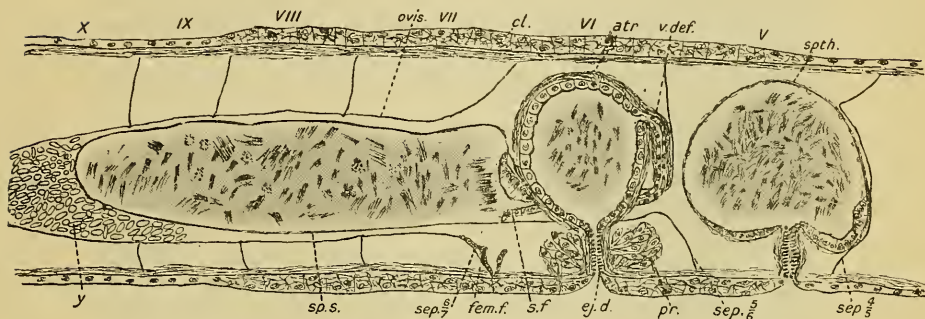
When such specimens were kept under observation for three days, their anterior portions, containing the genital organs,

separated off as a sort of cocoon, while the hinder part of the animal lived for some time, but was unable to regenerate and ultimately died. It appears, then, that death ensues after the full attainment of the sexual phase, and the cocoon is probably the whole anterior region of the worm which has been separated off.

BRANCHIODRILUS HORTENSIS Stephenson.

Many examples of this species were collected during the last two weeks of October and the first two weeks of November, from the mud at the bottom of a pond at Sikandra; and of these nine were found to possess fully developed sexual organs (text-fig. 3).

Text-figure 3.



Genital region of *Branchiodrilus hortensis*. The atria lie one on each side of the sperm-sac, and the vas deferens is seen running internal to the atrium of its side; the funnel lies far behind in the sperm-sac. *Pr.*, prostate; the remaining letters as before. \times ca. 125.

The clitellum occupies segments v.-viii.; it is opaque white in the living animal. It is about .05 mm. thick; the cells have a coarse reticular structure, the meshes of the network being clear spaces; the nuclei are indistinct. The clitellum is absent between the spermathecal openings and on the ventral surface in the anterior portion of segment v. It is formed after all the other sexual organs have been developed.

The testes had disappeared in all the specimens examined.

The sperm-sac may reach as far back as segment xix. or xx., in two cases to xxvi.; usually it extended to xviii.

The male funnels are within the sperm-sac, some distance behind its mouth, and here they nearly fill up the available space. Their lips are everted, and their outer margin is attached to the wall of the sperm-sac. The width of the funnel is 90μ ; its cells are high and ciliated, with nuclei at their base. The cells and cilia of the upper lip seem to be taller than those of the lower.

The vas deferens is 30μ in diameter; it is a fairly long tube, about .2 mm. in length, and consists of two parts, a posterior

longitudinal and an anterior ascending portion. The longitudinal portion passes forwards from the funnel, and is about 105μ in length; its last part lies over the upper wall of the sperm-sac. The tube then bends upwards; the vertical ascending portion, about 90μ in length, lies just behind septum 5/6. In a few specimens the longitudinal portion formed a distinct curve towards the ventral body-wall before rising to be continued into the ascending portion. The cells lining the vas deferens are about half as high as those of the funnel, have oval nuclei at the base, and are without distinct cell-outlines; there is a thin covering of muscle-fibres outside the epithelium. The tube enters the atrium on its anterior face much above the middle; the ascending part of the duct as it opens into the atrium is surrounded by a thick coat of muscle-fibres, continued onto it from the muscular covering of the atrium; the change from the cubical cells of the duct to the columnar cells of the atrium is sudden.

A bundle of muscle-fibres directed upwards from the ventral body-wall is attached to the ascending portion; and a few fibres connect it above to the dorsal body-wall behind septum 5/6.

The atrium is a large pear-shaped body, taking up nearly the whole length of its segment, $230-240\mu$ in height and about 190μ in length antero-posteriorly; in only one specimen were spermatozoa seen in its interior. The epithelium is columnar, the cells 18μ by 15μ , with oval nuclei lying at the base; outside the epithelium is a thick coat of circular muscular fibres.

The ejaculatory duct, about 78μ in length, opens at the top of a tubular depression of the ventral body-wall about 42μ in depth, and is capable of being everted, when it projects slightly as a short pseudo-penis. The epithelium of the duct consists of columnar cells with elongated nuclei, and has a thick investment of muscle-fibres.

The "prostate" consists of a large mass of pear-shaped cells around the ejaculatory duct; the cells contain granular protoplasm and a large oval or rounded nucleus near the base. A few muscle-fibres surround and enter the mass of cells from the ventral body-wall. There are a few blood-vessels around the atrium and prostatic cells. Two bands of muscle-fibres, arising from the setal sac, are attached to the atrium behind. The male opening lies internal to and at the level of the ventral setae about the middle of segment vi.

The ovisac, formed by the backward bulging of septum 6/7, and, as usual, enveloping the sperm-sac, is filled with a large mass of spherical and elliptical yolk granules about $7-15\mu$ in diameter. The ova are fairly large, and lie in two or three masses.

The female funnel, about 45μ in height, lies over the lower portion of septum 6/7 near the ventral parietes in segment vi. No female opening was seen.

The large spermathecae occupy the fifth segment. The ampulla

is somewhat heart-shaped, or ovoid and notched below where the duct arises. Its size varies somewhat: in height it may be from 207 to 270 μ , its length antero-posteriorly rather greater and its width rather less. The ampullæ are filled with spermatozoa, and are so large as nearly to fill up the whole segment, the remaining organs occupying only a small space below their contiguous inner walls. The part of the ampulla anterior to the duct is somewhat bulged downwards, and is lined with columnar cells; the part of the wall behind the duct is lined with cubical cells, which gradually decrease in height as they pass upwards on the posterior wall; the rest of the ampulla is lined by a very thin epithelium of attenuated cells whose outline is quite indistinct. Outside the epithelium there is a thin coat of circular muscular fibres—the only part of the wall visible over a large portion, on account of the thinness of the epithelium. The spermathecal duct leaves the ampulla below, nearer its anterior wall, and is about 130–140 μ in height, including the depression of the body-wall where it opens to the exterior. The duct is narrow above and below, but somewhat swollen in the middle; its epithelium consists of narrow columnar cells having elongated nuclei. There is a thick covering of muscle-fibres outside the epithelium. The spermathecal opening lies internal to and at the level of the ventral setæ of the fifth segment, some distance behind septum 4/5.

The penial setæ are the modified ventral setæ of segment vi. They are two or three in a bundle, somewhat hooked at the distal end, which is not bifid. In length they are about 132 μ ; the shaft consists of a distal narrow portion about 36 μ long, and a proximal stouter part 96 μ long; there is no distinct nodulus, but the distal narrow portion is bent outwards and thus not in a straight line with the proximal segment (text-fig. 1).

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