

ON *PISIONE COMPLEXA*, N.SP. FROM THE SANDY BEACH, MADRAS.¹

By K. H. ALIKUNHI, M.Sc.

(From the University Zoological Research Laboratory, Madras.)

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INTRODUCTORY AND HISTORICAL.

Collections from coarse sand near low water level of the Madras beach revealed the abundant occurrence therein of a Pisionid which proved to be an undescribed species. Two other Pisionids, *Pisionidens indica* (Aiyar and Alikunhi, 1940, 1943) and *Praegeria gopalai* (Alikunhi, 1941) have been previously recorded from the same area.

Hartman (1939) dealing with the family Pisionidae, considers that since the type of the genus *Praegeria*—*P. remota* Southern—resembles the type of the genus *Pisione*—*P. oerstedii* Grube—the retention of the genus *Praegeria* is unnecessary. *Praegeria remota* Southern becomes only a valid species of *Pisione*—*P. remota* (Southern). Hartman further finds that *Pisione germanica* described by Augener (1924) from the North Sea is identical with *Pisione remota* (Southern) from Ireland. Augener (*loc. cit.*) has already shown that *Pisione contracta* Ehlers, from Peru, is identical with *P. oerstedii* Grube. Hartman therefore concludes that 'two valid species are known to occur, *Pisione oerstedii* Grube from Peru, Ceylon and New Zealand, and *Pisione remota* (Southern) from Ireland and the North Sea'. To this he adds a new genus *Pisionella* which differs from *Pisione* in the possession of a median cirriform antenna at the anterior margin of the prostomium, three pairs of cirriform cirri, and the longitudinal series of spinelets on the setae tips and in the absence of the acicular setae from the buccal segment. Aiyar and Alikunhi (1940) created a third genus for an entirely different form under the name *Pisionella*, but owing to Hartman's genus having priority, have since changed it to *Pisionidens* (Aiyar and Alikunhi, 1943).

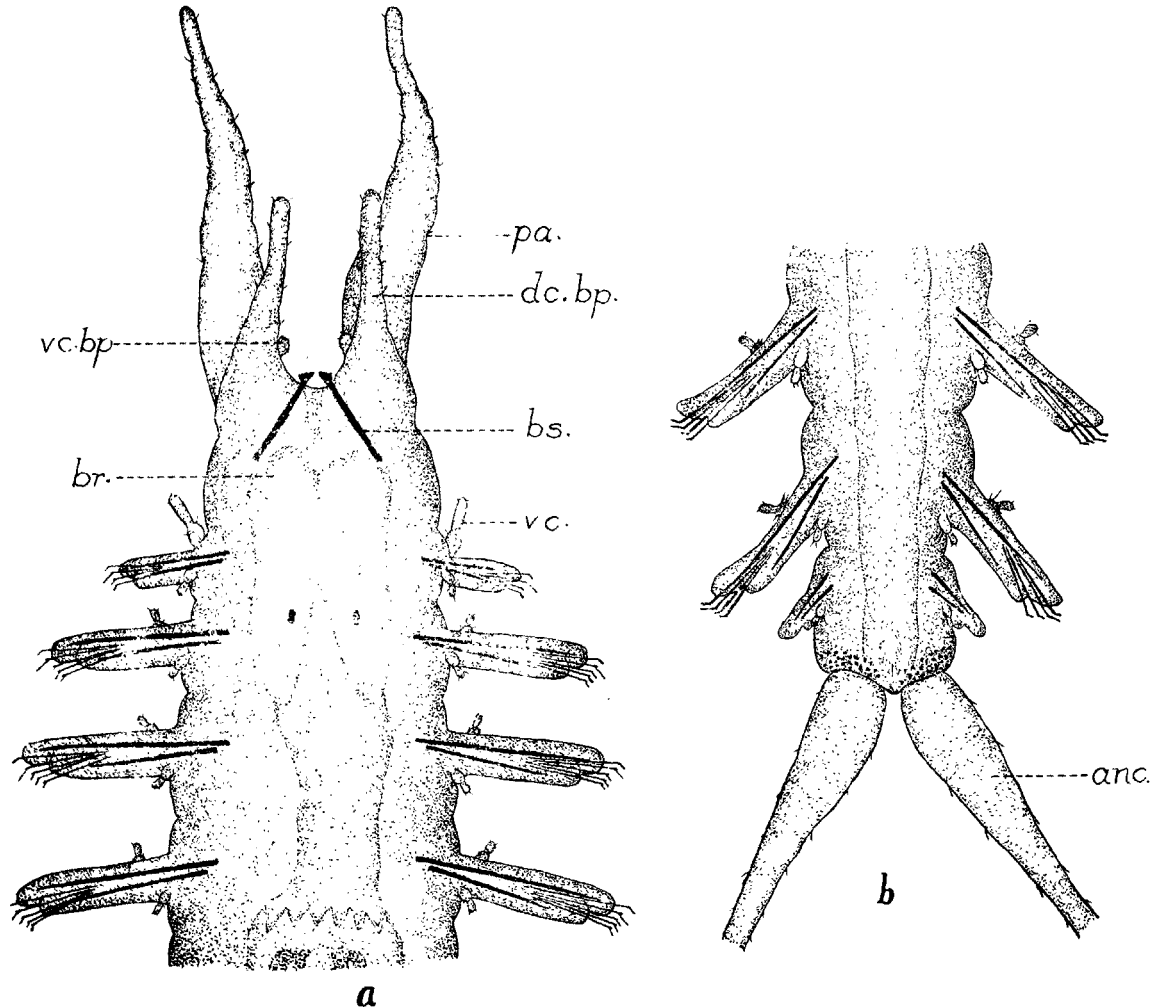
Hartman's paper was not accessible to me when my account of *Praegeria gopalai* was published in 1941. Since then I have had occasion to go through his paper and I am now convinced that it is unnecessary to retain the genus *Praegeria*. *P. gopalai* thus becomes *Pisione gopalai* (Alikunhi), and it forms the third valid species

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of the genus. The present form also belongs to the genus *Pisione* and it possesses all the peculiar features characterising the family Pisionidae as illustrated in the accounts of *Pisionidens indica* and *Pisione gopalai*. However, it shows a number of features peculiar to itself and in the following pages I propose to describe it as a new species under the name *Pisione complexa*.

EXTERNAL CHARACTERS.

The worms are comparatively large and measure about 10 to 25 mm. in length in the mature condition. The number of segments varies from 70 to 100 or even



TEXT-FIG. 1. *Pisione complexa*, sp. nov.

(a) Anterior end of the worm showing the cephalic appendages; body slightly contracted; drawn from specimen in spirit. $\times 120$.

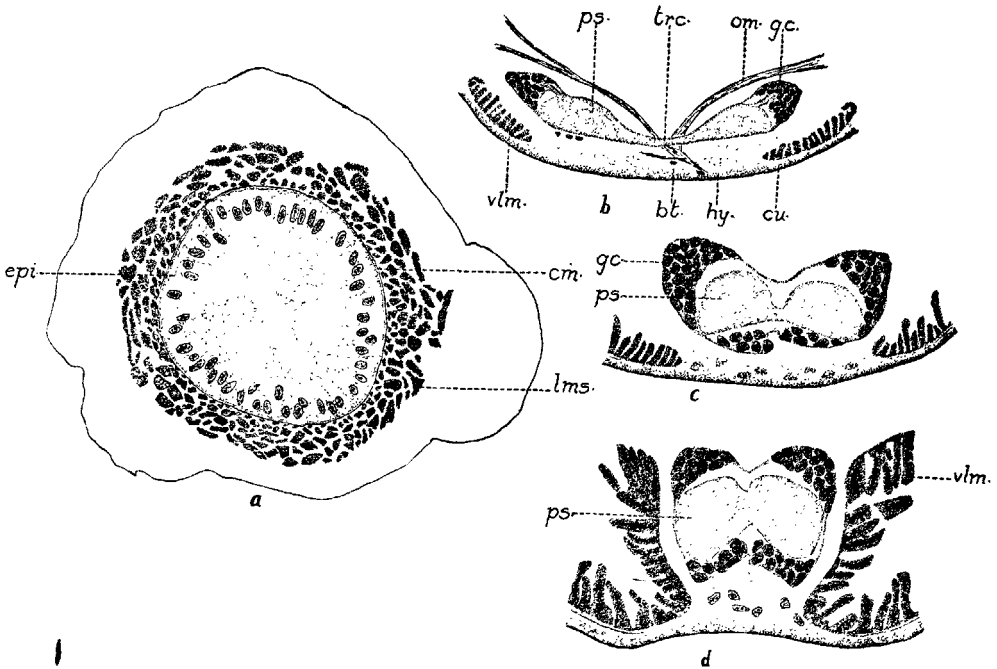
(b) Posterior end of the worm; from life. $\times 200$.

anc., anal cirrus; br., brain; bs., buccal spine; dc. bp., dorsal cirrus of buccal parapodium; pa., palp; ec., ventral cirrus; vc. bp., ventral cirrus of buccal parapodium.

more. The cephalic appendages are similar to those of *P. gopalai*. The ventral cirri of the first pair of parapodia are only slightly elongated (Fig. 1a). The buccal spines are devoid of any serrations at the tip and are shorter than those of *P. remota*. A pair of eyes are present attached to the brain. Parapodia are comparatively smaller than those of *P. gopalai*. The setigerous lobe is bifid at the tip where there are two papillae, the larger one of which becomes broader and foliaceous towards the posterior region. The setigerous support is identical with the same in *P. gopalai* and consists of two acicula and five setae in each foot, excepting in the first and the last four or five, in each of which there are only four setae. The body tapers considerably towards the posterior extremity. The anal segment is quite simple and resembles that of *P. remota* (Fig. 1b). It is a button-shaped structure to the posterior extremity of which is attached a pair of long anal cirri swollen at the base and gradually tapering to the tip. At the posterior extremity of the anal segment are aggregated a few coiled hypodermal glands the secretions of which serve for adhesion, like the secretion from the pygidial glands of *P. gopalai*.

BODY-WALL.

Transverse sections are usually circular in outline but may be slightly compressed dorso-ventrally (Figs. 5 and 8). The epidermal layer is very thin and the nuclei stain lightly. The circular muscle layer is inconspicuous and can be made out only



TEXT-FIG. 2. *Pisione complexa*, sp. nov.

- (a) Transverse section of the stomach showing the musculature. $\times 267$.
 (b) Nerve area— anterior region. $\times 400$.
 (c) Nerve area—stomach region. $\times 400$.
 (d) Nerve area—middle region. $\times 400$.

bt., basement tissue; cm., circular muscle; cu., cuticle; epi., epithelium; gc., ganglion cells; hy., hypoderm; lms., longitudinal muscle; om., oblique muscle; ps., punctated substance; trc., transverse connective; vlm., ventral longitudinal muscle.

at the sides. The longitudinal muscle bands form an almost complete wall to the body cavity. They are better developed than in the other species and in the ventral bands the folded edges usually come very near each other, thereby approaching the condition in *Pisionidens indica* (Aiyar and Alikunhi, 1940). The nerve area is located between the folded inner edges of the ventral longitudinal muscle bands (Fig. 2*b-d*). The coelomic membrane lining the body cavity is extremely thin. Epidermal glands are mostly confined to the sides of the body.

Coelomic corpuscles, oval, circular or disc-like, float freely in the coelomic fluid in the anterior segments.

ALIMENTARY CANAL.

The various regions of the alimentary canal are similar to those described in *P. gopalai*, except for some minor differences such as the greater development, generally, of the musculature, and especially the presence of a layer of circular muscles internal to the longitudinal muscles of the stomach and intestine, and the larger size of the cells constituting the inner epithelium of the stomach (Fig. 2*a*).

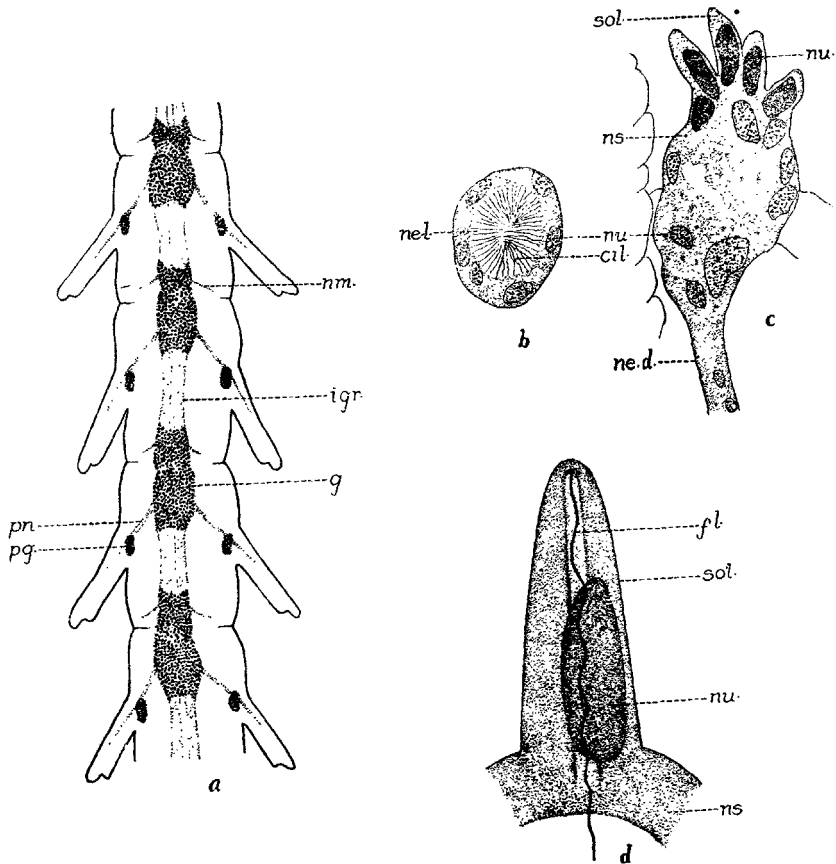
NERVOUS SYSTEM.

The posterior lobes of the brain are short and extend only into the anterior part of the third setigerous segment (Fig. 1*a*). The anteriorly directed dorsal cirri of the buccal parapodia are supplied by a pair of slender nerves which arise from the anterior part of the oesophageal commissures. The origin of these nerves from the oesophageal commissures is evidence that these structures are modified parapodial cirri. The commissures fuse together in the second setigerous segment, to form the ventral nerve cord. In the anterior segments the two cords stand rather apart and at the ganglionic swellings there are distinct connectives in between (Fig. 2*b*). In the front region the ventral longitudinal muscles being poorly developed, the nerve area is very broad. In these segments a part of the ganglionic swelling lies on the inner aspect of the muscle band, on each side (Fig. 2*b*). Externally to the nerve cord occur the basement tissue, the thin hypoderm and the cuticle. It is difficult to make out the circular muscles in this area. The oblique muscles are powerfully developed and are inserted into the basement tissue below the nerve cords. At the level of the stomach the nerve cords come closer together and the punctated substance in each half assumes a semi-circular outline (Fig. 2*c*). The ventral longitudinal muscles are better developed here and in consequence the nerve area is deeper and narrower than in front. This condition is maintained in the hinder regions of the body also.

The nerve cord has lost the primitive condition of being in contact with the hypoderm. In the absence of the circular muscle coat in the nerve area *P. complexa* resembles the Glyceridae (*Glysera capitata*) wherein 'the great external circular muscular layer ceases before reaching the nerve area, so that externally the latter has only the hypoderm and the specially developed cuticle' (McIntosh, 1877). But in *G. capitata* the ventral longitudinal muscles form an arch over the nerves, thereby differing from the condition in *P. complexa*.

As in *P. gopalai*, the nature of the ganglionic swelling in the ventral nerve chain is peculiar. Examined in the living condition, under the microscope, each segment shows a big ganglionic enlargement, the major part of which lying in the segment to which it belongs, while a small part extends into the segment in front. This is clearly seen in whole mounts (Fig. 3*a*). A pair of podial nerves arise from the main portion of the ganglionic swelling and each of these enlarges into a prominent ganglion situated at the base of the parapodial lobe and then proceeds further into the latter.

A second pair of very slender nerves originate from the anterior portion of the ventral ganglia and innervate the muscles of the body-wall.



TEXT-FIG. 3. *Pisione complexa*, sp. nov.

- (a) Diagrammatic representation of four segments of the worm, from a whole mount stained in Delafield's haematoxylin, showing the ventral ganglia and their disposition.
 (b) Transverse section of the nephridial swelling. $\times 1200$.
 (c) Longitudinal section of the nephridial swelling and solenocytes. $\times 1200$.
 (d) Reconstructed diagram of a solenocyte.

cil., cilium; *fl.*, flagellum; *g.*, ganglion; *igr.*, interganglionic region; *ne.d.*, nephridial duct; *ne.l.*, nephridial lumen; *nm.*, nerve to muscle; *ns.*, nephridial swelling; *nu.*, nucleus; *pg.*, parapodial ganglion; *pn.*, parapodial nerve; *sol.*, solenocyte.

EXCRETORY SYSTEM.

Excretory organs in the form of paired nephridia are present in all the segments excepting the anterior five. The nephridium ends internally in the form of a swelling projecting into the body cavity from the posterior corner of the segment. In general structure it shows close resemblance to that of *P. gopalai*. The first two or three pairs of nephridia are larger with a larger number of solenocytes. The nephridial swelling has a spacious cavity which is richly ciliated and is almost circular in cross-section (Fig. 3b). The cells forming the nephridial swelling are large, though their boundaries cannot be clearly made out. The nuclei stain but lightly.

The solenocytes are situated on the anterior face of the nephridial swelling and have a crowded appearance (Fig. 3c). Each solenocyte has a broad base and tapers gradually to the tip. There is no distinction into a cell-body and a flagellum-carrying tube (Fig. 3d). There is a narrow lumen and a long flagellum, attached to the wall at the distal extremity, works rapidly down the lumen. The nucleus is elongated and has a baso-lateral position (Fig. 3c). The nephridial duct at its commencement describes one or rarely two spirals before piercing the septum to open at the base of the ventral cirrus.

Nothing is known of the nephridia of *Pisione oerstedii* and *Pisionella hancocki*. In *Pisionidens indica* and *Pisione gopalai* the nephridial system has been shown to be very much like that described by Goodrich (1900) for a number of phyllodocids. In *Pisione remota* (Southern) which also occur in the Madras beach—an account of the nephridia and the reproductive organs of which will form the subject matter of a separate communication—the nephridia are similar to those in other pisionids. As the foregoing account shows, in *Pisione complexa* also there is that phyllodocid type of nephridium. There is the same nephridial swelling from which solenocytes arise. Only the solenocytes lack a flagellum-carrying tube distinct from the cell-body—a condition which is mentioned for the first time. It will thus be seen that the affinities of the Pisionidae are altogether with the Phyllodocids, Nephthyds and the Glyderids, in the structure of their excretory organs.

The nephridia undergo modifications in the genital segments and these will be described in detail along with the reproductive organs.

REPRODUCTIVE SYSTEM.

Male.

Testis.

The males are usually smaller than the females. The reproductive organs are highly localised and are invariably found to be developed after the 30th segment. In the mature male the testes are developed in a varying number of separate segments. The smallest mature specimen examined was one with 38 setigerous segments and this had a single pair of testes situated in the 32nd segment. In a specimen with 55 setigerous segments testes were developed in segments 32 and 44, while another, 18 mm. long and with 98 segments, had 6 pairs of testes occurring in segments 32, 44, 56, 61, 71 and 77 respectively. In yet another with 100 setigerous segments only four pairs of testes were developed and these occurred in segments 35, 38, 58 and 82 respectively. The arrangement therefore is not regular.

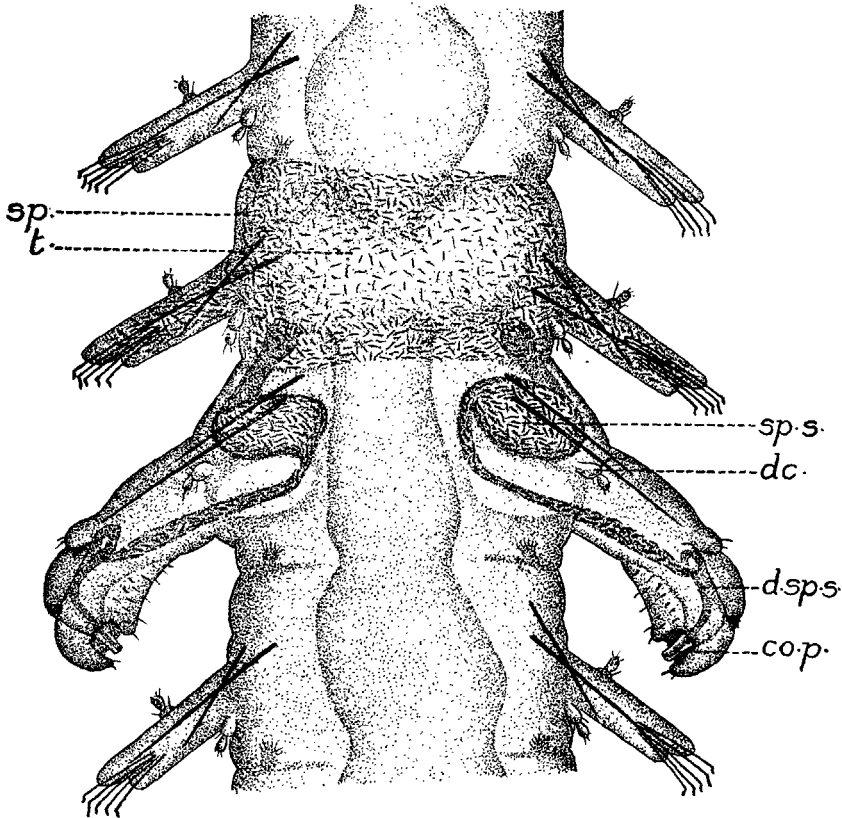
The worms probably mature when they develop about 38 to 40 segments and then there is only a single pair of testes, situated in one of the posterior segments. Growth in length continues by the addition of fresh segments at the posterior end and along with this at varying intervals fresh testes groups are also developed. This, therefore, accounts for the occurrence of individuals with varying number of testes, sperm-sacs and copulatory organs in various stages of development. The maximum number of testes developed depends upon the length of the worm, but of the numerous specimens examined none has been found to possess more than six pairs of testes.

Each testis is invariably confined to a single segment which always precedes the one carrying the sperm-sacs (Fig. 4). It originates as paired cell proliferations attached to the septum and has a thin outer limiting membrane, which is clearly visible in transverse sections. In the later stages of maturity the testis becomes smaller and less conspicuous owing to the liberation of sperms.

Sperm-sacs.

Each testis is invariably followed in the next segment by a pair of sperm-sacs which become associated with a pair of copulatory organs (Fig. 4). The nephridia

in the testis-bearing segments are inconspicuous and a pair of genital funnels of about 4 to 5 times the size of the unmodified nephridial swelling becomes associated



TEXT-FIG. 4. *Pisone complexa*, sp. nov.

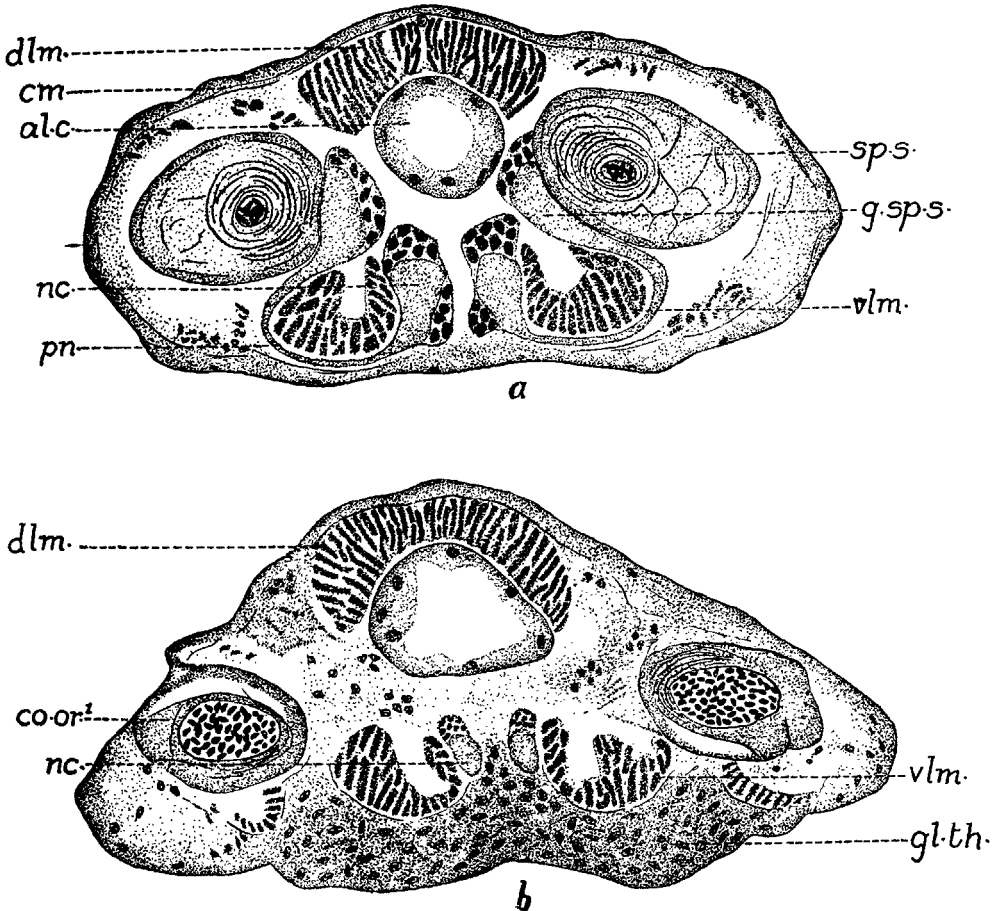
Genital segments of a mature male showing the distribution of the reproductive organs. $\times 200$.

co.p., copulatory process; *dc.*, dorsal cirrus; *d.sp.s.*, duct of sperm-sac; *sp.*, sperm; *sp.s.*, sperm-sac; *t.*, testis.

with them. When fully formed these funnels are situated close to the body-wall, on the dorso-lateral aspect of the reduced nephridial swelling (Figs. 4 and 6). They are deep spoon-shaped structures with ciliated margins. The solenocytes of the nephridia of these segments get shorter and inconspicuous and can be distinguished under high magnification only by the characteristic downward lashing movement of their flagella. The nephridial lumen is narrow and the genital funnel opens into it at the point where it pierces the septum. There is no loop or coiling of the nephridial duct before its piercing the septum and in this respect also the nephridia of the testis-bearing segments differ from those of other segments. Behind the septum, the nephridial duct runs down to a short distance and then sharply bends upwards and forwards to get enlarged into a narrow thin-walled sac (Figs. 4 and 6). Proceeding further, it narrows and bends sideways and backwards and running almost straight down, enters the copulatory organ. It is interesting to note that this second descending portion corresponds to the highly spacious, muscular, second

dilatation in the sperm-sac of *P. gopalai*. Therefore, in this form even though there is a well developed sheath of circular muscles surrounding the hind portion of the sperm-sac, the nephridial duct is not distended into a second saccular portion. In living specimens the surrounding muscles are not very conspicuous. The liberated sperms are thickly packed inside the saccular portion. The interior of the sperm-sac is powerfully ciliated. The external duct on entering the copulatory organ enlarges imperceptibly, forms a loop about the middle of its course and is continued to its external opening situated at the tip of a papilla (Figs. 4 and 6).

The parapodial nerves are stout and at the outer edge of the ventral longitudinal muscles they turn inwards and enlarge into a pair of ganglia which come to be situated close to the ventral wall of the sperm-sacs (Fig. 5a). From each of these ganglia a stout nerve—corresponding to the nerve to the parapodial lobe—is given



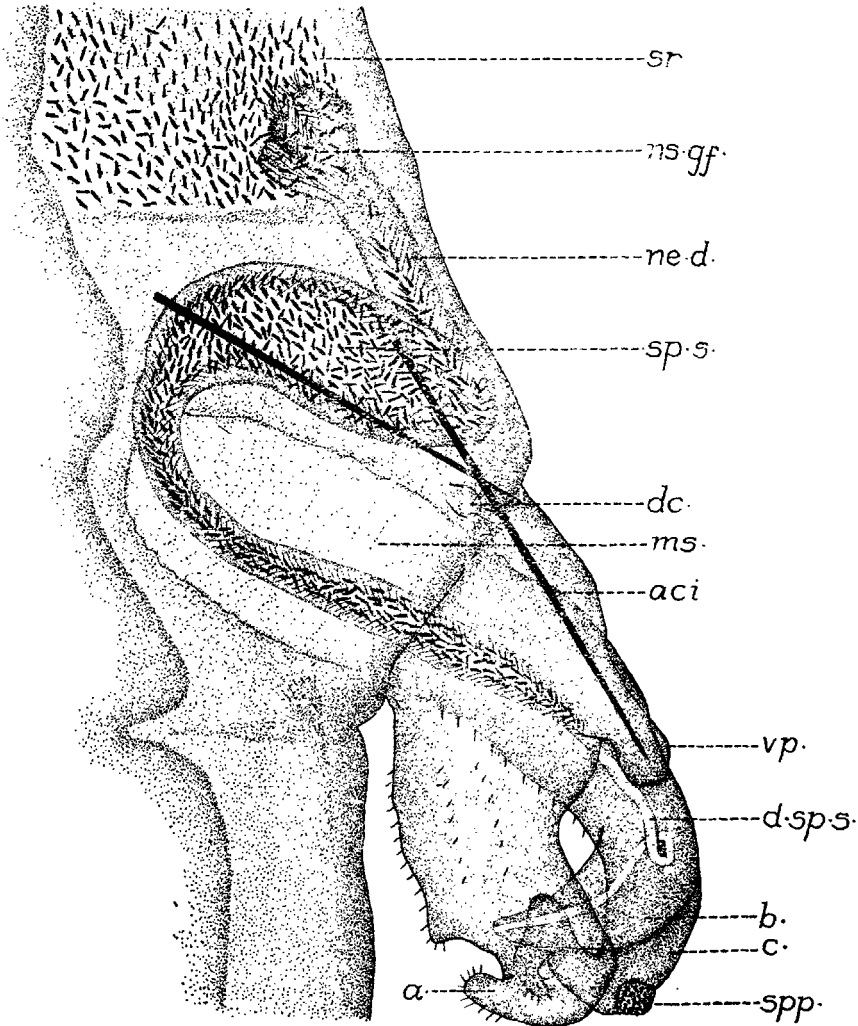
TEXT-FIG. 5. *Pisione complexa*, sp. nov.

- (a) Transverse section of the sperm-sac segment of the male showing the nerves arising from the ventral nerve cord going to the sperm-sacs. $\times 540$.
 (b) Transverse section of the posterior part of the sperm-sac segment showing the glandular pad on the ventral side. $\times 540$.

alc., alimentary canal; *co.or.*,¹ proximal part of copulatory organ; *dlm.*, dorsal longitudinal muscle; *gl.th.*, glandular thickening; *g.sp.s.*, ganglion to sperm-sac; *nc.*, nerve cord. (Other letters as in previous figures.)

off which proceeds to the tip of the copulatory organ along the dorsal aspect of the sperm-sac. The presence of this nerve throws light on the homology of the copulatory organ discussed in a subsequent section.

In the sperm-sac bearing segment the longitudinal muscles are very much reduced. The dorsal and ventral bands are strictly confined to the dorsal and ventral sides respectively (Fig. 5a). The alimentary canal is pushed dorsad and is in the form of a narrow tube. The available coelomic space is thereby increased and it is mainly occupied by the sperm-sacs. Towards the hinder part of this segment a fairly thick glandular pad or thickening is formed ventrally (Fig. 5b). The cells



TEXT-FIG. 6. *Pisione complexa*, sp. nov.

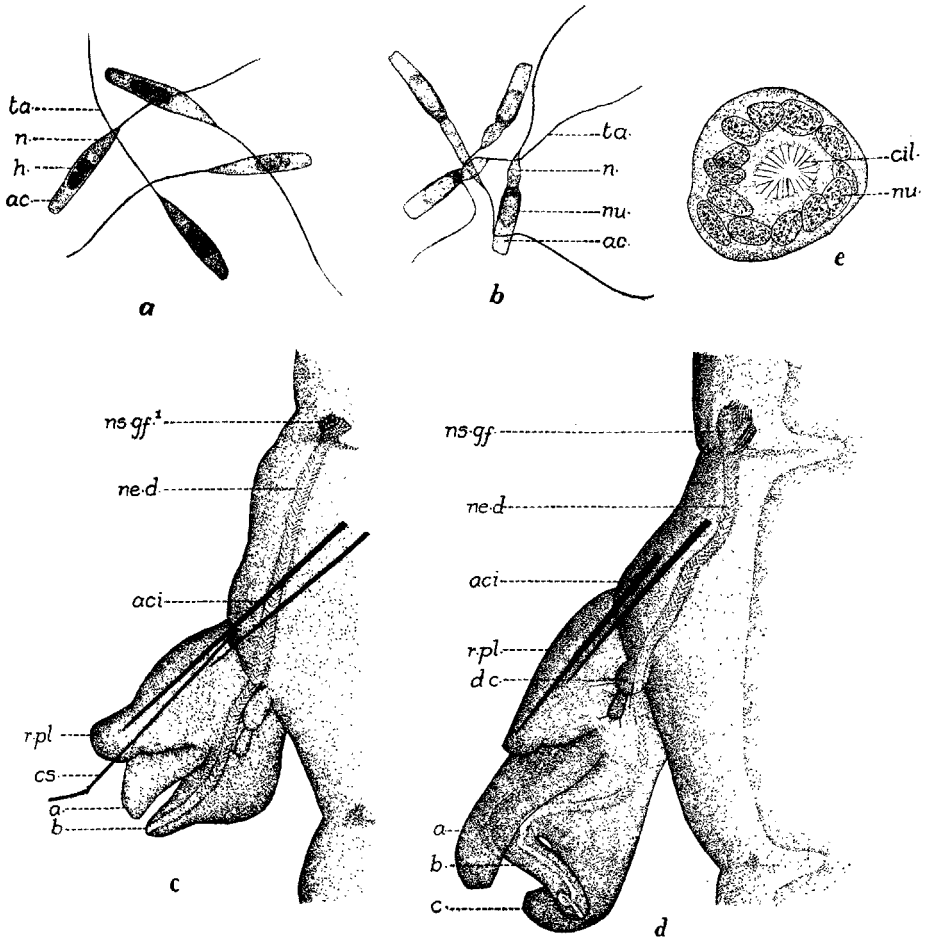
Magnified drawing of sperm-sac and copulatory organ of one side. $\times 400$.

a.b.c., processes of copulatory apparatus; *aci.*, aciculum; *ms.*, muscular sheath; *ns.gf.*, nephridial swelling and genital funnel; *spp.*, spinous papilla; *vp.*, vestigial papilla. (Other letters as in previous figures.)

forming this thickening have darkly staining nuclei of various shapes. It is probable that this glandular pad in *P. complexa* serves at least to some extent the function of the ventral suckers in *Pisionidens indica* and are probably epidermal glands specially developed for purposes of adhesion during copulation.

Copulatory Organs.

The number of copulatory organs varies from 1 to 6 pairs, or even more depending upon the number of pairs of sperm-sacs and testes developed. Each



TEXT-FIG. 7. *Pisione complexa*, sp. nov.

- (a) Sperms from the male—fresh preparation. $\times 1200$.
 (b) Sperms from the male—from a smear stained in Delafield's haematoxylin. $\times 1200$.
 (c) Copulatory organ—early stage. $\times 267$.
 (d) Copulatory organ—later stage. $\times 267$.
 (e) Transverse section of the nephridial duct in the genital segment of a developing male. $\times 1200$.

ac., acrosome; cs., compound seta; h., head; n., neck; ns.gf.¹ nephridial swelling and rudiment of genital funnel; r.pl., reduced parapodium; ta., tail. (Other letters as in previous figures.)

copulatory organ is an elongated, non-retractile structure, about twice the size of the normal parapodium. In the segment carrying the copulatory organs the dorsal cirrus of the parapodium remains unmodified. The main lobe of the parapodium has undergone extreme reduction and is represented only by a minute vestigial papilla (Fig. 6). Its setigerous support consists of only two acicula, compound as well as simple setae being absent. From the ventral aspect of the base of the vestigial papilla representing the parapodial lobe, is given off a broad foliaceous structure ending in a curved papilla provided with palpcils (*a* in Fig. 6). As will be shown later, this papilla represents a highly modified ventral cirrus, and probably forms part of the copulatory apparatus. Such a structure is entirely lacking in *P. gopalai*. From the ventral aspect of this papilla arises a finger-like process which divides distally into two. The ventral one of these processes is elongated and carries at its end a minute, retractile, spinous pad just behind the tip (*c* in Fig. 6). This spinous structure corresponds to the muscular papilla so conspicuous in the copulatory organs of *P. gopalai*. The efferent duct of the sperm-sac runs through the dorsal process to open to the outside at the tip of a conical papilla (*b* in Fig. 6).

Sperms.

Sperms when liberated pass into the coelomic space of the testis segment. They do not usually spread into the neighbouring segments. To the naked eye the testis and sperm-sac bearing segments appear white. The sperms are non-motile. Each sperm is slender and elongated and measures about 40 to 45 microns in length, including the flagellum. The acrosome is prominent but blunt and measures about 4μ (Fig. 7*a*). The nuclear portion is oval in outline, more refractile than the acrosome, and measures about 3-4 microns. The neck portion following the head is longer, about 5 microns, narrow and tapering to the posterior extremity whence commences the long flagellum measuring about 28 to 30 microns.

Higher magnification reveals that at the commencement of the neck there is a minute granule, probably representing the centriole.

DEVELOPMENT OF SPERM-SACS AND COPULATORY ORGANS.

The formation and differentiation of the adult sperm-sacs and copulatory organs have been followed by examining a number of developing male specimens, in various stages of maturity. Figures 7*c* to 7*e* illustrate some of the stages. In the segment that is destined to develop the sperm-sacs in the adult, the parapodium is very much reduced. An unmodified ventral cirrus is absent while the dorsal cirrus remains unchanged. Two acicula and one compound seta support the reduced parapodial lobe. From the ventral aspect of this lobe arises a conspicuous structure which is sub-divided into two, the dorsal one of which, judging from its position, seems to correspond to the ventral cirrus of the unmodified parapodium (*a* in Fig. 7*c*). The rudiments of the genital funnels are present in association with the nephridia, in the form of a few large cells. Cilia are not yet developed. The nephridial duct is considerably thicker than in other segments and running down almost straight, enters the base of the newly formed ventral structure and proceeding forwards opens at the tip of the ventral of the two processes into which it is divided (*b*, Fig. 7*c*). Testis is developed in the preceding segment but is small and does not fill the compartment. At this stage there is no trace of the sperm-sacs in the segment carrying the developing copulatory organs.

In another specimen, slightly more advanced in development, the setigerous lobe in the segment carrying the developing copulatory organ was smaller and had lost the compound seta which was present in the previous stage. The dorsal of the two processes—the modified ventral cirrus—mentioned in the previous stage is elongated (*a*, Fig. 7*d*). The ventral process, now very much enlarged in size, splits into two by developing a broad, slightly curved structure ventrally (*c*, Fig. 7*d*).

The papilla carrying the efferent duct of the nephridium has further elongated and is bent at right angles (*b*, Fig. 7*d*). The rudiments of the genital funnels are larger and have developed a central lumen with a crown of vibratile cilia. This lumen has attained communication with the nephridial duct as in the fully mature specimen. The nephridial swelling is very much reduced. The nephridial duct has a straight course in the body and there are no saccular expansions in its course. Immediately behind the septum the nephridial duct is prominent and broad. It has a thick wall, the constituent cells being highly protoplasmic. The nuclei are large and closely situated and form an almost complete ring surrounding the central ciliated lumen (Fig. 7*e*). In the region behind, the duct gradually becomes narrow and thin-walled. On entering the copulatory organ it has assumed a similar course as in the mature specimen. The testis in the preceding segment has undergone further development and cell division and occupies the major portion of the segmental chamber.

In the next stage the developing copulatory organ assumes the adult condition. The nephridial duct behind the septum makes a bend and enlarges into a saccular portion as in the adult. Behind this saccular portion a thick muscular covering is developed around the nephridial duct. The testis is fully developed and a few sperms have been liberated into the body cavity. The essential parts of the adult sperm-sac have now been differentiated, and in the next stage which is the fully ripe condition, more sperms are liberated from the testis and are carried down the nephridial duct to be stored in the sperm-sac. It is, therefore, quite clear that the adult sperm-sac is formed by the differentiation of the simple straight nephridial duct. The latter thus becomes the sperm-duct and in so functioning as a passage for the genital elements to the exterior, has undergone some transformatory changes, and in this feature the Pisionids are more specialised than most of the polychaetes.

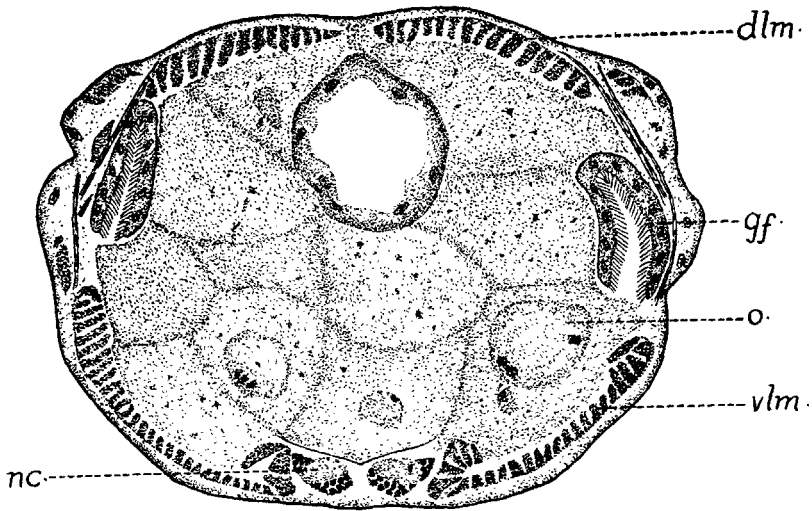
A comparison of the structure of the copulatory organ with that of *P. gopalai* is instructive. As has been described, the entire structure in *P. gopalai* is comparatively simple, there being developed only a single papilla, carrying the efferent duct of the sperm-sac, besides the reduced parapodial lobe which later gets completely suppressed leaving no trace behind. In the corresponding stage in *P. complexa* we get two additional processes making the structure more complicated. Again, a compound seta is never present in the reduced parapodium of the segment carrying the copulatory organs in *P. gopalai*, at least when the worm is attaining sexual maturity for the first time (see below).

The structure of the developing copulatory organ suggests its probable origin. In the segment which is destined to become the genital segment the parapodium consists of a dorsal cirrus, a highly reduced main lobe, and the developing copulatory organ. The ventral cirrus has undergone considerable modification. Normally, in the non-genital segments the nephridial opening is situated below the ventral cirrus. Now, judging from the position of the developing copulatory apparatus in relation to the parapodium, it seems probable that the finger-like process immediately ventral to the reduced main lobe of the parapodium corresponds to the ventral cirrus (*a* in Figs. 6, 7*c* and 7*d*). The body-wall below this modified ventral cirrus, and probably partly from its base, undergoes a projection, lengthens out and splits into two processes, the dorsal one of which carries to its end the nephridial duct, while the ventral one undergoes further differentiation, becomes flattened and develops a pad of spinous processes. We must, therefore, regard the copulatory organ proper as being formed almost entirely *de novo* from the side of the body-wall. This, together with the adjoining highly modified ventral cirrus may be said to constitute the copulatory apparatus.

Female.

The ovaries are confined to the second-half of the body and usually 6 to 20 pairs of them are developed. They are highly localised and definitely paired in origin.

Each pair is situated within the confines of a single segment. Transverse sections reveal the presence of an extremely thin membrane covering the ovary. In the ripe individual the segmental chamber is fully occupied by ova and distinction between the right and the left ovary is lost. With the expansion of the ovarian mass the great longitudinal muscle bands are extremely reduced. The hypodermal layer is very much attenuated, and the alimentary canal is considerably narrowed and pushed very much dorsad (Fig. 8).



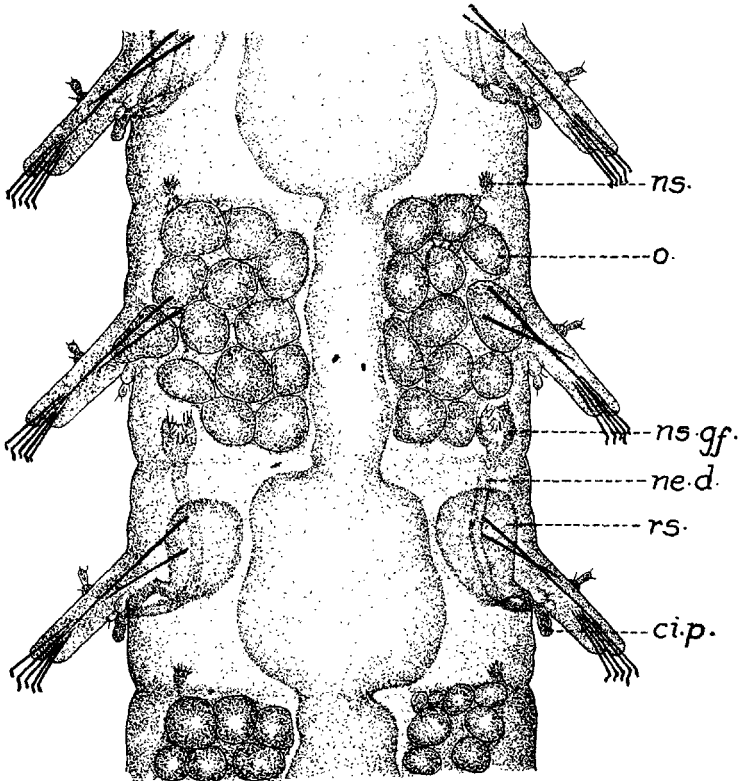
TEXT-FIG. 8. *Pisione complexa*, sp. nov.

Transverse section through the posterior region of an ova-bearing segment of a ripe female. $\times 540$.

gf., genital funnel; o., ovum.

The reduction in the thickness of musculature and its partial atrophy is a common feature in the sexually mature forms of various polychaetes and such changes have been noted in considerable detail in several families (Fage and Legendre, 1927). In the present case, however, the musculature only undergoes reduction without any actual destruction. Fage and Legendre (*loc. cit.*) and Caullery and Mesnil (1898) have given detailed accounts of the modifications undergone by the digestive tube in the genital segments of a number of polychaetes. In certain forms in the sexually ripe individuals the alimentary canal is so pressed by the gonads that the inner sides of the intestine are applied one against the other, without leaving any space in between, with the result that the digestive tube is no longer functional and the animal cannot nourish itself during this period. In such forms as *Eulalia punctifera* when the segments are relieved of the genital elements the alimentary canal assumes its normal condition and becomes functional again. But there are others in which hystolysis takes place in the contracted region of the alimentary canal, which, in consequence, gets disintegrated and the animal does not survive oviposition. Instances in point are met with in certain Phyllodocids, Glycerids, Cirratulids, etc. In *P. complexa* even though the digestive tube in the genital segments is greatly attenuated, there is no fusion of the intestinal walls and it is probable that the organ carries on its usual function, at least in a restricted sense, throughout the sexual period. The worm survives oviposition and resembles *Eulalia punctifera* in that the digestive tube assumes its normal dimensions when the genital elements are shed.

Each ovarian segment is invariably followed by another in which ova are never developed but in which a pair of receptacula seminis are situated (Fig. 9). The ovaries and the receptacula seminis, in other words, have an alternating arrangement. In a worm with 60 setigerous segments 8 pairs of ovaries were developed in segments 41, 43, 45, 47, 49, 51, 53 and 55 respectively, while in the intervening segments were situated the corresponding pairs of receptacula seminis. The number and position of the receptacula seminis, therefore, depend upon the number and position of the ovarian groups. In this localisation of a certain number of segments for the ovaries and an equal number solely for the receptacula seminis, *P. complexa* differs from *P. remota* but closely resembles *Pisionidens indica*, even though in the latter each ovarian group extends through more than one segment.

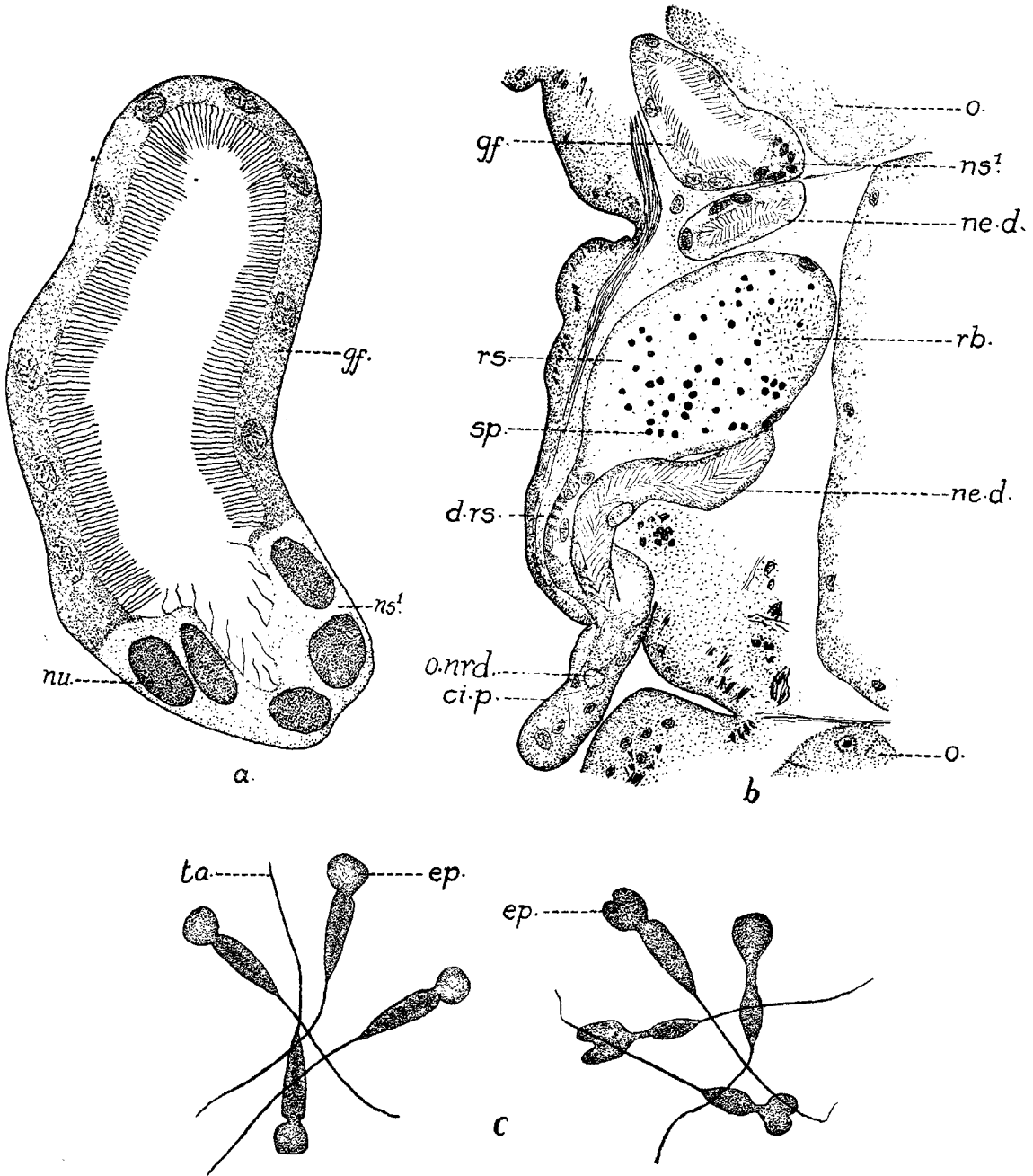


TEXT-FIG. 9. *Pisione complexa*, sp. nov.

Genital segments of a ripe female showing the arrangement of the various structures.
× 200.

ci. p., cirriform process; *rs.*, receptaculum seminis.

The parapodia of the segments in which the receptacula seminis are situated, are unmodified. In ripe individuals from the ventral aspect of the base of the parapodium of the particular segment, arises an elongated papilla with the nephridio-receptacular aperture (see below) at its base (Figs. 9 and 10*b*). This process is larger than either of the parapodial cirri. It corresponds, in the female, to the highly developed copulatory organ proper of the male. Such a structure is absent in *P. gopala*. In *Pisionella hancocki* Hartman (1939) figures such a cirriform structure attached to the ventral basal aspect of the parapodial lobe.



TEXT-FIG. 10. *Pisione complexa*, sp. nov.

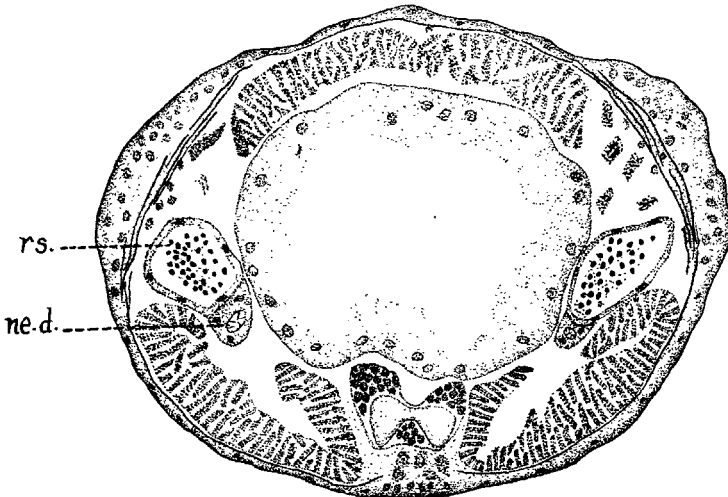
- (a) Longitudinal section of the nephridium and the associated genital funnel of a ripe female. $\times 1800$.
 (b) Longitudinal (horizontal) section—female—showing the opening of the nephridial duct into the distal portion of the receptacular duct. Note the cirriform structure at the base of which the duct opens. $\times 600$.
 (c) Sperms from the receptaculum seminis of the female. $\times 1800$.

d.rs., duct of receptaculum seminis; *ep.*, expanded portion; *ns.*¹ reduced nephridial swelling; *o.nrd.*, nephridio-receptacular opening; *rb.*, refractile bodies.

A pair of well-developed genital funnels are present in each of the ova-bearing segments (Fig. 9). These funnels in the fully mature condition are about twice the size of those in the males and become closely associated with the nephridia. The relative position of the nephridium and the genital funnel is the same as in the male. Each funnel is in the form of an inverted spoon with the two edges of it projecting beyond above the highly reduced nephridial swelling (Fig. 8). In other words, the funnel is hood-like with the concavity directed ventrally. A transverse section passing through the anterior region of the funnel is in the form of an inverted *V* or *U*, while further behind the gap between the limbs of the *U* gets closed up by the nephridial swelling (Figs. 8 and 10a). The wall of the genital funnel is formed of a single layer of large ciliated cells, the nuclei of which stain lightly. In the nephridial portion the nuclei of the solenocytes take deep stain (Fig. 10a and b).

It may be pointed out that nephridia still function in the testis and ova-bearing segments, though in a reduced condition, and the ciliated funnel is only grafted on to the dorso-lateral aspect of the nephridial swelling—a condition which generally resembles the relationship between corresponding organs in the Phyllodocidae and the Glyceridae. In this connection the relation between the genital funnel and the nephridium in *Pisionidens indica* may be recalled in that the nephridia in the genital segments do not undergo any reduction in size or difference in structure, but that only a large conspicuous ciliated funnel is grafted on to it for the conduction of the genital elements to the exterior—a condition much the same as in Phyllodocids. The reduction in size of the nephridial swelling in the genital segments of *Pisione gopalai* has already been described (Alikunhi, 1941). This reduction in size and the highly inconspicuous nature of the protonephridium when associated with the genital funnel make these composite structures in their fully developed condition, superficially resemble the typical nephromixia of the Hesionids, Spionids, Syllids, etc., but the presence of solenocytes functioning at least in a reduced condition along with the genital funnel is proof that their real structure is on the Phyllodocid plan.

After piercing the septum the nephridial duct runs down between the alimentary canal and the receptaculum seminis. At this stage it is wide and powerfully ciliated. Further down it proceeds along the ventral aspect of the receptaculum seminis and gets narrow (Fig. 11). The receptaculum seminis is a closed thin-walled sac,



TEXT-FIG. 11. *Pisione complexa*, sp. nov.

Transverse section passing through a pair of receptacula seminis. $\times 300$.

usually containing spermatozoa. It is not ciliated internally. The cells in its wall at the closed end are extremely flattened. The sac opens to the exterior, as already mentioned, at the parapodial base on a special process (Figs. 9 and 10*b*). The external duct is thick-walled, the constituent cells being large and protoplasmic. It is further thickened by the development of a thin layer of circular muscles. Some distance in front of the external opening of the duct of the receptaculum seminis, the nephridial duct joins the former, with the result that there is only one common external aperture—the nephridio-receptacular aperture, as in the case of *P. gopalai* (Fig. 10*b*).

Structure of Sperms within the Receptaculum Seminis.

The presence of copulatory organs in the male and receptacula seminis containing sperms, in the female, point to the occurrence of copulation, but I have not been able to observe the process. The arrangement of the accessory reproductive structures, however, suggests that the process might be similar to that described in *Pisionidens*. But genital papillae or suckers are absent in *Pisione* and adhesion between the copulating individuals may be brought about by the large flattened process of the copulatory organ, with the help of the specially developed glands on the ventral surface of the sperm-sac bearing segments, and probably also by the modified ventral cirrus.

Mature females are invariably found to have been inseminated. The sperms inside the receptacula seminis have undergone metamorphosis and as in *P. gopalai*, the new modifications have apparently taken place after their transference to the receptaculum seminis. The sperms when pressed out of the receptaculum seminis are seen to be loosely held together by a sticky fluid, probably secreted by the wall of the receptaculum seminis itself. There is no formation of spermatophores.

Each sperm has developed a broad, more or less circular anterior extremity which is marked out from the succeeding region by a distinct constriction (Fig. 10*c*). The expanded portion probably represents the acrosome. The nuclear portion that follows the constriction is spindle-shaped, with the tapering neck region behind. The sperm measures up to 28 μ , of which 8 microns are made up by the anterior expansion and the head, while the rest, by the flagellum.

Certain variations from the structure described above are often met with in some individuals. It might be that these variations are progressive stages before the final condition is arrived at, but I have no confirmatory evidence on this point. In some of these sperms the broad anterior expansion is found to be partly bisected by a deep median indentation (Fig. 10*c*). In some others the constriction that follows the anterior expansion is very much elongated in the form of a connective. The median indentation may be absent in some. In sections the nuclear portion takes a deep stain and is circular in outline. The sperms have undergone a distinct reduction in size as compared to those from the sperm-sacs of the male. How this is effected and how the changes are brought about, I am not now in a position to explain.

The changes undergone by the sperms while inside the receptacula seminis, in *P. gopalai*, have already been described in detail. The sperms being non-motile the remarkable transformations undergone by them after copulation seem to have something to do with ensuring fertilisation by the development of an adhesive structure which would enable them to adhere firmly to the surface of the female gamete at the first contact.

In this connection it may be pointed out that somewhat similar instances of 'metamorphosis' of the spermatozoa in the receptacula seminis have been noted in Molluscs. Ikeda (1929) has described an instance of metamorphosis of spermatozoa in the Japanese slug *Phylomicus bilineatus* Benson. Similar instances have also been observed by the same author in other slugs like *P. fruhstobei*, *Eulimax flavus*, *Limacella agrestis*, etc. In *P. bilineatus*, Ikeda says that the mature

spermatozoon stored in the receptaculum seminis has no tail, but only a sperm-head and that the metamorphosis takes place in the atrium after copulation. During metamorphosis the head and the middle piece get separated from each other by the formation of a slender filament in between, and finally by the breaking of this filament the connection between the two is severed. Ikeda is finally inclined to believe, on the evidence of his own experiments, that this metamorphosis of the spermatozoon is probably related to the mechanism of fertilisation rather than to the prevention of self-fertilisation in the hermaphrodite molluscs.

In the case of *P. complexa* the sexes being separated the prevention of self-fertilisation as a reason for metamorphosis of the spermatozoon does not arise. In this case also it is therefore probable that these changes, as suggested before, have something to do with the mechanism of fertilization. A detailed and comparative study of the metamorphosis of the spermatozoon in this and other Pisionids is being made in the hope that it would be possible to throw some light on this exceedingly interesting phenomenon.

The ova are large, greenish in colour and in the fresh condition have big transparent nuclei. Sections show that in the early oocyte the nuclear portion is more or less clear with a circular central darkly staining nucleolus. In the fully formed ovum the nuclear portion takes a greyish tinge with iron hæmatoxylin, is rather opaque, and usually has an inner clear area. The nuclear membrane is present. The nucleolus has undergone division and portions of it have already been passed into the surrounding cytoplasm. The details of these changes being outside the scope of the present paper are not now considered.

When mature, the ova are liberated into the body cavity and are attracted towards the wide mouth of the genital funnels by their ciliary action. They are then taken into and passed down the enlarged nephridial duct. As in the case of *P. gopalai*, it is believed that as the ova pass down the nephridial duct the sperms that are stored into the receptaculum seminis are also sent down which would enable them to get fertilized just prior to, or immediately on their being extruded.

CHANGES AFTER LIBERATION OF GENITAL ELEMENTS.

The account that is given below is based upon observations of the changes occurring in a number of individuals after the shedding of the genital elements. These observations have been put together in a connected form for the sake of convenience and continuity, though it should be understood that all these changes have not been observed in a single form. Most of the observations have been confirmed from worms directly obtained from the beach at different times.

Female.

Sexually mature specimens are obtainable throughout the year. When ripe females are kept in the laboratory for a week or so the ova are completely shed. Breeding takes place continuously and when the genital elements are shed, probably after a short resting period, the individual develops another set of genital elements. It is, however, found that in either sex the accessory structures such as the copulatory organs, sperm-sacs, genital funnels and receptacula seminis are gradually lost after the shedding of the genital elements.

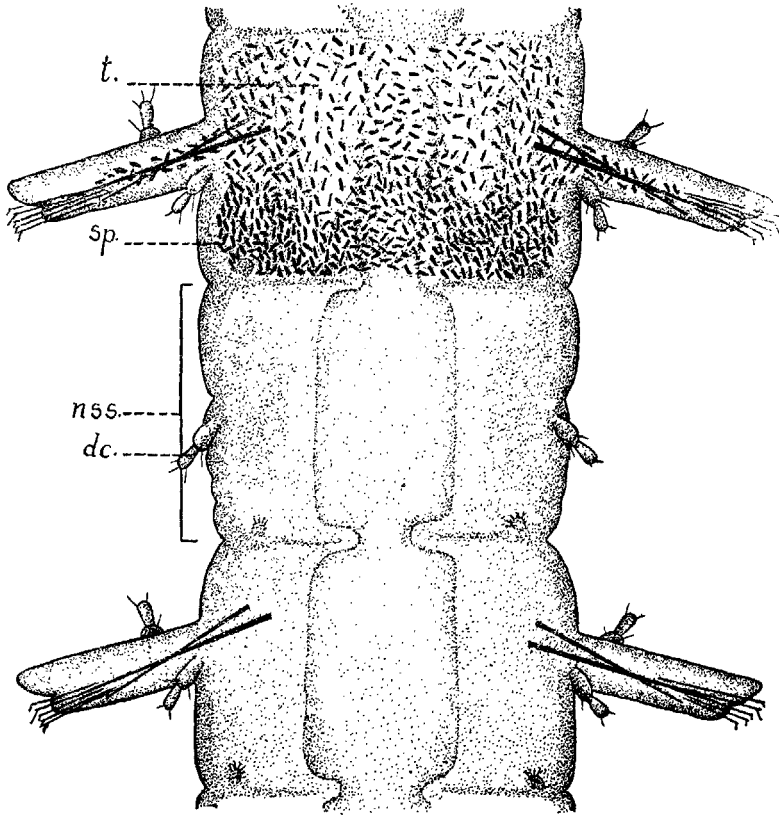
With the shedding of the ova the genital funnels begin to get smaller. The receptacula seminis, some of them still containing sperms, are pushed nearer the anterior septum and their external ducts show signs of hystolysis. The nephridial duct is unchanged. The ovary has returned to its immature condition and is now represented by a small mass of tissue.

Prolonged captivity results in further changes. The genital funnels disappear, with the result that the nephridial swellings of the respective segments stand out prominently. There is no trace of the receptaculum seminis. The nephridial duct

now communicates independently to the exterior. The cirrus like structure, developed from the parapodial base is absent. The worm now looks just like an immature one, with just a trace of the ovary.

Male.

A similar series of changes take place in the males also. The testis becomes reduced and is often represented by minute groups of cells surrounded by sperms. The genital funnels are also very much reduced and portions of the sperm-sac show signs of histolysis. Copulatory organs still retain their outer shape, but internally histolysis has commenced. At a later stage the genital funnels, sperm-sacs and copulatory organs are all completely shed and the dorsal cirri alone persist in the genital segment (Fig. 12). The segment is now nonsetigerous, the acicula also having been cast off.



TEXT-FIG. 12. *Pisione complexa*, sp. nov.

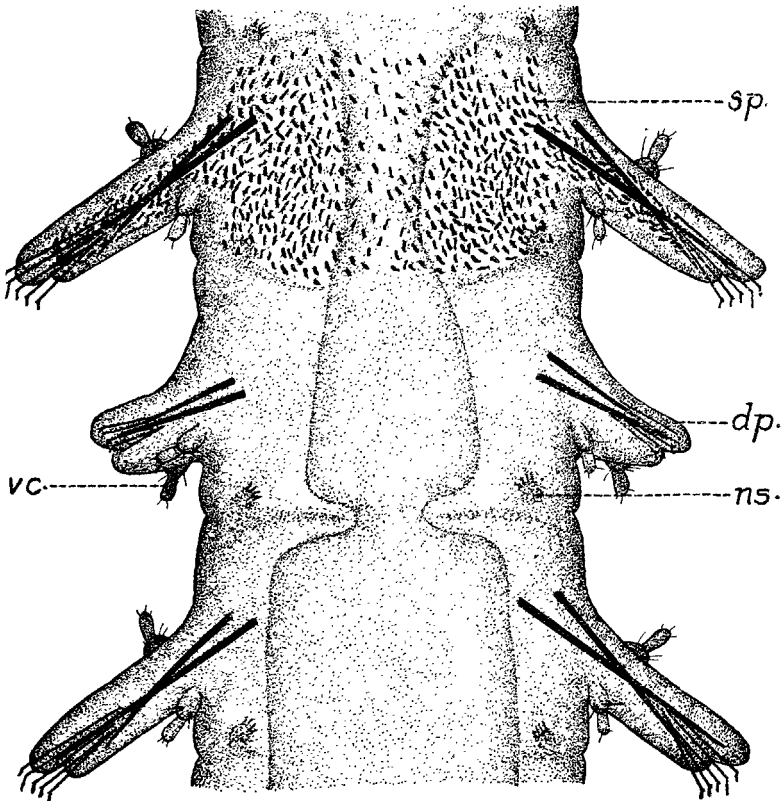
Middle segments of a male worm showing the genital segments from which sperm-sacs and copulatory organs have been dropped off. $\times 200$.

nss., non-setigerous segment.

The changes undergone by the nephridial duct are evidently more complicated here than in the female. Since in the male genital segment the external opening of the nephridial duct—modified into the genital duct—is situated at the tip of the copulatory organ, the question arises whether the nephridia in these segments

function when the sperm-sacs—mere dilatations of the nephridial duct—and the copulatory organs are undergoing profound histolysis which culminate in their entire loss. With the disintegration of these structures it is clear that the nephridial duct behind the septum is also lost. It is therefore, highly probable that the nephridia in these segments do not function when histolysis is taking place and that the nephridial swelling which now stands alone in the testis bearing segment develops a fresh external duct by the time the ventral cirrus and parapodial lobe are developed in place of the copulatory organs (see below).

At a later stage fresh parapodia begin to develop from either side of the segments in which sperm-sacs and copulatory organs were present (Fig. 13). Ventral cirrus is formed. Compound as well as simple setae are developed in the newly formed parapodial lobe. These parapodia get further elongated and in this condition differ in no way from the parapodia of the non-genital segments. The testis is very minute and like the ovary has returned to the undeveloped condition. The segment still contains some worn out sperms in the coelom (Fig. 13). The ultimate fate of these sperms is not fully known. Since the nephridia have no direct internal opening, with the disintegration of the genital funnels and sperm-sacs these sperms are effectively prevented from being taken to the exterior. In the males, therefore, the accessory organs are lost before the genital elements are completely shed.



TEXT-FIG. 13. *Pisione complexa*, sp. nov.

Middle segments of a male that has cast off the sperm-sacs and other accessory structures, developing ordinary parapodia. Note the condition of the fresh parapodia and the presence of sperms in the preceding segment. $\times 200$.

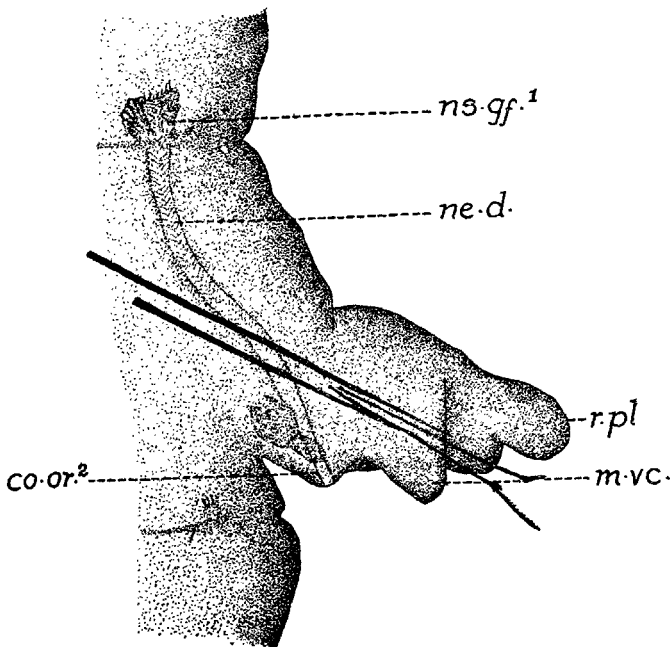
dp., developing parapodium.

Changes of a similar nature taking place in *Pionosyllis neapolitana* have been described by Goodrich (1930), but in the male segments of this hermaphrodite syllid the testes are always in a well-developed condition, apparently ready to produce more sperms. This is not the case in *P. complexa*. Whether any change takes place in the sperm-sacs of *Pionosyllis* is not known.

It is clear that the reproductive organs are lost and renewed probably several times in the life history of an individual. But the time taken for the development of the first set, the interval between its loss and the formation of the next, are points that remain to be elucidated.

SECOND GENERATION OF REPRODUCTIVE ORGANS.

The second set of reproductive elements is developed in the very same segments which contained the first, since the remnants of the ovary or the testis as the case may be, are present in those segments in an inactive condition. In the male, the parapodia of the segment following the testis, though resembling the others, were smaller and their bases were considerably swollen (Fig. 14). The ventral cirrus was absent and in its place there was a broad papilla with a swelling at the base. On this swelling was situated the external aperture of the nephridium, the course and appearance of which were similar to those of the nephridial duct in the developing copulatory organ (Fig. 7c). The absence of the ventral cirrus at this stage and the presence of an enlarging papilla in its place definitely shows that the normal ventral cirrus is being modified during the formation of the copulatory process. Also the swelling at the base of the enlarging ventral cirrus supports the conclusion arrived at previously that the actual copulatory process is formed as a prolongation of the



TEXT-FIG. 14. *Pisione complexa*, sp. nov.

Regeneration of copulatory organ—early stage, viewed from the ventral aspect.
× 400.

co.or.,² developing copulatory organ; m.vc., modified ventral cirrus.

body-wall at the base of the ventral cirrus. A cluster of cells was attached to the nephridial swelling, evidently forming the rudiments of the genital funnel. The parapodial lobe was supported by two acicula, one simple and one compound seta. The testis was still minute and undeveloped. It is to be observed that in the formation of the copulatory organ during the second generation the well developed parapodium gets gradually shorter and shorter, at the same time casting off its setae until only the two acicula remain. In this feature it differs from its first formation when a well developed parapodium was not present in its place. The succeeding stages are similar to those in the first formation of the copulatory organ described above. It is to be noted that a few sperms of the first generation are still present in the segmental chamber. It is possible that these are senile sperms which might be absorbed during the increased metabolism that is taking place as a result of the formation of fresh genital funnels, sperm-sacs and copulatory organs. It is also suggestive that the retention of sperms in the testis segments till the regeneration of the reproductive organs incidently forms one of the means by which the animal finds material to meet the demands of the increased metabolic activity, and might probably explain what would otherwise seem a waste of energy on the part of the worm in producing more sperms than are actually used during copulation.

Might not these phenomena indicate some sort of rejuvenation of the worm accompanying the replacement of the once functional reproductive organs by an entirely new set? Recent researches on rejuvenation in other animals seem to indicate that this is not altogether impossible.

SPECIFIC CHARACTERS.

Pisione complexa n.sp.

Worms 10 to 20 mm. long, with 70 to 100 or more segments, cephalic region identical with that of *Pisione gopalai*, anal segment semi-circular, without anal glands, one to six or more pairs of testes and a corresponding number of genital funnels, sperm-sacs and copulatory organs in the male, each testis invariably confined to a single segment, sperm-sac with only one saccular expansion, copulatory apparatus of extraordinary complexity, parapodial lobe in the sperm-sac bearing segment highly reduced and supported by two acicula in the adult and by two acicula and one compound seta in the earlier stage, the ventral cirrus modified into a broad papilla, ventral glandular pad on the sperm-sac bearing segment, sperms non-motile and of large size, females having up to 20 pairs of ovaries, enclosed in membranous coverings, arranged in alternating segments, the intervening segments occupied by the paired receptacula seminis, genital funnels in every ova-bearing segment, parapodia of segments carrying receptacula seminis unmodified, nephridio-receptacular aperture situated on a special cirriform structure ventrally to the parapodium, and sperms within the receptacula seminis also non-motile but with highly expanded anterior extremity.

Locality: Madras beach, India.

ACKNOWLEDGMENTS.

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