

Reprinted from JOURNAL OF THE NEW YORK ENTOMOLOGICAL SOCIETY,
Vol. LVI, March, 1948, pages 25-41.

THE MALE GENITALIA OF EPHEMERIDA
(MAYFLIES)

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The male reproductive systems of seven species of mayflies were studied in order to form a basis of comparison between their morphology and that of those European species studied by Quadri (1940), as well as that of several species of *Stenonema* used by Needham *et al.* (1935).

Pickles (1931) devoted a portion of his paper to the rôle played by the mayfly alimentary canal in the later development of the male reproductive system, and discussed the theories of Palmen (1884) and Fritze (1888).

Spieth (1933) described both the development of and the adult form of the male genitalia of Ephemera, and used the genitalia as one of the factors in a study of the phylogeny of some mayfly genera.

Needham *et al.* (1935) presented a brief description of the morphology of the male genitalia and the internal anatomy of the male reproductive system of mayflies, including the histology, based on studies of nymphs and adults of *Stenonema vicarum*, with four other species of this genus used for comparison.

Snodgrass (1936) described the morphology of the male genitalia of mayflies and gave considerable material on the embryology of these parts and their homologies in other insect groups. He included diagrams of the male genitalia of *Ephemera simulans* and *Blasturus cupidus* and went into detail concerning the muscles of the accessory genitalia.

Quadri (1940) used data obtained from five species of mayflies, *Chleone dipterum*, *Ephemera vulgata*, *Heptagenia* sp., *Rhithrogena* sp., and *Batis* sp. Under the heading of "Adult Organs," he described the organs of the reproductive system, discussed their histology, and gave a description of the penes and accessory genitalia. Under "Development," he considered the embryology and development of both internal and external genitalia.

In the present investigation, mature nymphs, subimagoes, and imagoes of the following species were used:¹

Hexagenia limbata occulta Walker
Siphonurus quebecensis Provancher
Ephemera guttulata Pictet
Heptagenia hebe McDunnough
Blasturus cupidus Say
Stenonema rubromaculatum McDunnough
Isonychia bicolor Walker

The insects were studied by means of reconstructions from serial sections, whole mounts, and microdissections. All specimens had been fixed and preserved in 70–95 per cent ethyl alcohol, and it was found that four hours of post-fixation in Zenker's Fluid resulted in somewhat better preparations. Both nymphs and adults were treated in the same way.

The abdomens were removed just posterior to the fifth segment and then post-fixed. It was occasionally found necessary to squeeze air out of the abdomens to prevent them from floating on the surface of the various fluids used during imbedding. Dioxane was used for dehydration and the regular procedure for paraffin imbedding was followed. A 58–60° C. paraffin was found to give the best results. The blocks were then sectioned at 10–12 microns and the ribbons were fixed to the slides with the aid of albumin. After drying, the preparations were stained with a dioxane technique, Levy (1943).

EXTERNAL GENITALIA

The following is a general description of the morphology and histology of the male genitalia of *H. limbata occulta* imagoes. The general histological picture is the same in the other species except where differences are specifically mentioned, although the shape, size, and appearance of these structures may differ in each species.

¹ All the specimens used were from the collection of Dr. Herman T. Spieth, who identified all the material, and to whom I am indebted for invaluable suggestions and criticism during the progress of the work and the preparation of the manuscript. I would also like to thank Dr. James I. Kendall and Dr. Charles D. Michener for their and and criticism.

The penes of *H. limbata occulta* originate from the membrane between the dorsal part of the styliger plate and the paraprocts of the tenth sternum and appear as beak-like structures with their posterior ends curving medially. They are covered by a thick layer of cuticula which is heaviest at the basal region and on the dorsal side. The hypodermis of the penis is well developed in the imago.

The cuticula of the penes is apparently made up of a thick layer of exocuticle and a well developed epicuticle, although the tips of the penes seem to lack the latter. The endocuticle is either poorly developed or absent.

The styli or forceps of *occulta* originate from the ventrolateral parts of the styliger plate and are composed of a basal segment (which has the only muscles found in these structures), a long, slender subsegment, and two short terminal subsegments. Snodgrass (1936) called these "subsegments," claiming that they are not true segments since they lack muscles. The long first subsegment of *occulta* is laterally constricted along most of its length, so that it appears dumb-bell-shaped in cross-section. This condition is not found in any other species studied.

The medial surfaces of the styli are covered by many small mating glands, derived from the hypodermis, which project through the cuticula. These glands appear to have a thin layer of endocuticle and are easily ruptured. Spieth (1933) noted that the styli of *Blasturus* lack these glands, and this was found to be the case in *B. cupidus*.

INTERNAL GENITALIA

In *H. limbata occulta*, the muscles of the styli, as stated by Snodgrass (1936), attach at the ventrolateral wall of the anterior portion of the styliger plate and insert near the stricture between the basal segment and the first subsegment. The rest of the stylus, as stated by Needham *et al.* (1935), is filled with fluid containing blood corpuscles. There are also a number of small tracheoles.

The above description applies also to those structures in *Siphonurus quebecensis*, *Isonychia bicolor*, *Stenonema rubromaculatum*, *Heptagenia hebe*, and *Ephemera guttulata*. *Blasturus cupidus* shows some differences.

Spieth (1933) pointed out that the styli of *Blasturus* originate from the dorsal surface of the styliger plate, while this is not the case in most genera. He also noted that the styli of this genus lack the basal articulation found in the Heptageniidae and *Siphonurus*, and that the styli of *Blasturus* are only three-jointed.

The anterior attachments of the muscles of the styli in *B. cupidus* are on the ventrolateral wall of the styliger plate, as in other species studied. However, these muscles attach posteriorly on the dorsolateral wall of the posterior portion of the styliger plate, this in view of the fact that the basal segment is lacking in the styli of this species.

In *H. limbata occulta*, the dorsal portion of the basal two-thirds of each penis is almost completely filled by a heavy mass of muscle fibers, the ventral portion being occupied by the ejaculatory duct which extends to the tip. These muscles run obliquely in the penes so that their anterior attachments are on the lateral walls of the basal portions of the penes, and their posterior attachments are on the medial walls of the distal portions, although there are no attachments in or near the tips. Their probable action is to bend the penes medially, and to aid in the ejaculation of spermatozoa. The rest of the penis is filled with fluid containing spindle-like blood corpuscles.

These muscles first appear in the mature nymph, and may develop from undifferentiated cells seen in the developing penes of immature nymphs. It should be emphasized that these muscles are attached only within the penes, and have neither origins nor insertions on any other structure or part of the insect. No evidence was found to indicate whether or not they may have migrated to the penes from any other area.

These same muscles have also been found in mature nymphs, subimagoes, and imagoes of *Isonychia bicolor*, and the above description fits this species as well.

As shown in figures 5, 6, the penes of *Heptagenia hebe* and *Stenonema rubromaculatum* are quite different in appearance from those of *H. limbata occulta*, although the same penial muscles are found in both of these species. Some of the medial or posterior attachments of these muscles are located more distally than in *H. limbata occulta* and are found on the extreme postero-

medial walls. Since relatively more of the basal portions of the penes in these two species are joined together than is true in *occulta*, the muscles in the extreme basal regions are attached on the ventro-lateral walls, while the posterior attachments are on the medial portions of the dorsal walls. This condition exists only in the basal portions. The penial muscles do not have any attachments in the distal lateral processes of the penes of either of the two species.

The internal morphology of the penes of *Siphonurus quebecensis* is quite different from that of any other species studied. The presence of muscles in the penes of mature nymphs, subimagoes, and imagoes has been noted, but these muscles have different positions and may have different origins than those of the species previously discussed.

In mature nymphs of *S. quebecensis*, the penial muscles originate on the ventrolateral walls of the ninth sternum, posterior to the origins of the muscles of the styliger plate, and are attached on the dorsolateral walls of the developing penes. The developing styliger plate is found posterior to the origins of these muscles. These muscles are better developed in the subimago and in the imago. They originate on the ninth sternum walls just posterior to the most anterior part of the styliger plate and are attached to the dorsolateral walls in the middle portions of the penes. They have no attachments in either the basal or distal portions.

No undifferentiated cells, such as those seen in the penes of *H. limbata occulta* nymphs, were seen in the penes of near-mature, *S. quebecensis* nymphs. Unfortunately, there is insufficient evidence to indicate the embryological origin of these muscles in *quebecensis*.

The absence of muscles in the penes of mature nymphs, subimagoes, and imagoes of both *Ephemerella guttulata* and *Blasturus cupidus* has been noted. No evidence was found in *E. guttulata* of the existence of the basal arms or lateral prolongations of the penes, nor of the muscles described and figured as activating these structures in *E. simulans* by Snodgrass (1936).

In *H. limbata occulta*, the vasa deferentia appear to originate in the testes as a result of the coalescence of a number of small testicular tubules. They proceed posteriorly between the dorso-

lateral muscles of the abdomen and enlarge in the posterior portion of the eighth abdominal segment to form the seminal vesicles. These, distended with spermatozoa, fill almost all of the ninth segment and the anterior part of the tenth.

The seminal vesicles are joined together to form a single tube at the point where they empty into the ejaculatory ducts, in a manner shown in figure 1, which are at this point also joined together to form a single duct. This duct soon bifurcates, and the ejaculatory ducts terminate at the gonopores at the tips of the penes.

The ejaculatory ducts of *occulta* are enclosed by a thick layer of circular muscles and are lined by a flattened epithelial layer. The muscles are heaviest at the level of the basal portions of the penes, gradually decreasing and disappearing as the ducts near the gonopores.

The seminal vesicles of *occulta* are lined by a thin epithelial layer with invisible cell boundaries and elongate, granular nuclei. Enclosing this layer is an equally thin layer of circular muscles of the same type found in the ejaculatory ducts.

The structure of the seminal vesicles in *occulta* shows some variation, not only between individuals, but between the two vesicles of the same individual as well. Among the approximately thirty specimens of this species examined, however, all showed the same general characteristics.

Other workers have shown that there is a close relationship between the changes in the gut in the mature nymph and subimago and the changes in the reproductive system at this time. Palmen (1884) suggested that air is taken into the gut of the subimago, causing it to inflate, and that this pressure is responsible for histological changes in gut tissue.

Fritze (1888) claimed that the flattening of the epithelium and the dilation of the mesenteron is due to intake of water by the nymph shortly before the emergence of the subimago, the water then being replaced by air.

Pickles (1931) described the reduction of the epithelium of the alimentary canal and stated that the gut contents are expelled before the transformation of the alimentary canal commences. In his summary, he stated that extensive changes take place in

the structure of the gut in which both epithelium and musculature become reduced, forming a thin membrane in the imago. He claimed that no pressure exists in the gut until the tissue transformation has taken place.

In all species used in this study, the mature nymphs' guts did not contain any food material, indicating that they had ceased to feed at some previous time, nor was any distortion of the gut noted at this time. The movements of the gut at the time of the emergence of the subimago are apparently of great importance in the conformation of the parts of the reproductive system.

In the mature nymph, the genital tube is a straight, undifferentiated structure with neither folds nor pouches. When the mature nymph emerges to become the subimago, it takes in large gulps of air, which inflate the gut like a balloon as far posteriorly as the ilio-colic constriction, which acts like a valve. This violent expansion of the gut, in addition to the action of the abdominal muscles, serves to push the whole genital apparatus posteriorly. The testes are moved back to the seventh and eighth segments, and at the same time, are emptied of their spermatozoa, which pass through the vasa deferentia into the seminal vesicles. These, enormously distended, are forced posteriorly to occupy only the ninth segment and the anterior part of the tenth.

Such movement causes a number of changes in the relative position of the seminal vesicles, both in the horizontal and vertical planes. In the vertical plane, they are folded to form Z-shaped loops. Since the ninth segment is smaller and narrower than are the preceding segments, in almost all mayflies, this same pressure causes that region of the genital tube which includes the posterior parts of the seminal vesicles and the extreme anterior parts of the ejaculatory ducts to loop medially and anteriorly in the horizontal plane. The medial walls of each loop are brought together and break through, so that at this point there is a common ejaculatory duct receiving the contents of a common seminal vesicle. This series of changes is apparently completed in the subimago, and no further change has been noted in the imago.

The morphology of the genital tube in imagoes of *Isonychia bicolor* is different from that seen in *H. limbata occulta*. In *I. bicolor*, the seminal vesicles are separate and distinct structures

and there is no connection between them at any point. Similarly, there is no fusion of the anterior parts of the ejaculatory ducts, and the contents of each seminal vesicle pass into the ejaculatory duct on that same side.

Each seminal vesicle, as it enters the ninth segment, enlarges until it occupies about one-quarter of the abdominal cavity. At about the middle of the ninth segment, these vesicles curve ventrally to enter the penes. Some specimens show the Z-shaped loops seen in *H. limbata occulta* to a moderate degree, but most do not. The seminal vesicles are divided into compartments, at right angles along almost their entire length, by a series of septa, each of which is attached to the wall of the seminal vesicle by trabeculae. Each septum is apparently made up of a core of connective tissue with a thin epithelial covering. These septa can be seen developing in mature nymphs. They are moderately well developed in the subimago and very well developed in the imago. The function and action of these septa are not known.

The ejaculatory ducts of *I. bicolor* have a less well developed coat of circular muscles than do those of *H. limbata occulta*, and have a better developed epithelium with numerous small folds.

The seminal vesicles of *Siphonurus quebecensis* resemble those of *H. limbata occulta*, but there is no fusion of the anterior parts of the ejaculatory ducts, nor is there any connection between the seminal vesicles. The form taken by these structures is much the same as in *H. limbata occulta*. The vesicles fill more than half the abdominal cavity and are filled with spermatozoa.

The ejaculatory ducts of *S. quebecensis* narrow abruptly in the basal part of the penes and the circular muscles disappear at this point, so that the posterior portions of these ducts consist of epithelial tissue only.

The same form of the seminal vesicles occurs in *Ephemera guttulata* as in *H. limbata occulta*, except that the Z-shaped loops are not so well developed, nor is there any connection between any of the parts of the two genital tubes. Testicular and Malpighian tubules are frequently found in the lumina of the adult penes of this species. The histology of the seminal vesicles and ejaculatory ducts of *guttulata* is the same as that of *H. limbata occulta*, except that the epithelial layer of the seminal vesicles is better developed in *guttulata* and has folds that project into the lumen.

The looped form of the seminal vesicles is not so pronounced in *Stenonema rubromaculatum* as it is in *H. limbata occulta*, nor is there any connection between the two genital tubes. In occasional specimens, the form taken by the seminal vesicles of *rubromaculatum* resembles that of *I. bicolor* very closely.

The distal portions of the seminal vesicles of *S. rubromaculatum*, near where the ejaculatory ducts begin, are divided at right angles to their length by septa similar in appearance to those seen in *I. bicolor*. However, these septa are not so numerous in *rubromaculatum* and are restricted to the distal portions of the seminal vesicles. The histology of these septa is the same as that of *I. bicolor*.

The ejaculatory ducts of *S. rubromaculatum* have a well developed epithelial lining and a poorly developed musculature. These ducts have been pushed into the seminal vesicles for a short distance somewhat like a piston into a cylinder. The ducts narrow abruptly in the posterior parts of the penes and lose their musculature, as in *Siphonurus quebecensis*.

The morphology and histology of the seminal vesicles and ejaculatory ducts of *Heptagenia hebe* is the same as that of *S. rubromaculatum*. There is no connection between any of the parts of the two genital tubes. However, the septa in the seminal vesicles of *H. hebe* are not so numerous as those of *S. rubromaculatum*.

In *Blasturus cupidus*, the seminal vesicles, unlike the other species studied, are found in the middle of the eighth abdominal segment and are about four times the diameter of the vasa deferentia. In the middle of the ninth segment, they are collapsed and empty, of about the same diameter as the vasa deferentia. These slender tubes then proceed posteriorly and enter the basal portions of the penes to become the ejaculatory ducts. This condition has been found in six imagoes and two subimagoes and does not appear to result from fixation effects. There is no connection between the two genital tubes. It is to be noted that the seminal vesicles are not looped, nor do they have any pouches in *cupidus*, but are straight, unfolded tubes. Unlike the other species studied, they fill less than one-eighth of the abdominal cavity in the ninth segment, while the posterior collapsed portions occupy much less space.

The expanded parts of the seminal vesicles of *B. cupidus* consist of an extremely thin circular muscle sheath with a much flattened epithelium lining the tube. In the collapsed parts of the vesicles, the epithelium is thicker and appears to be better developed. The ejaculatory ducts have a well-developed epithelium with a fairly heavy layer of circular muscles in the basal portions of the penes, which gradually disappears as the ducts near the gonopores.

DISCUSSION

Considering the limited number of genera used in this study, it is inadvisable to conduct more than a brief discussion of its implications. Admittedly, the relationships of wings, gills, external genitalia, and mouthparts are more valuable than are the internal organs of the reproductive system in studies of mayfly phylogeny.

It is interesting to note that only in *Hexagenia* is there any connection between the paired genital tubes, and that this occurs in such a way that both the seminal vesicles and the ejaculatory ducts are involved. The similarity in the septa seen in the seminal vesicles of *Isonychia*, *Heptagenia*, and *Stenonema* indicates a close relationship between these three genera as mentioned by Spieth (1933). The morphology of the seminal vesicles of *Blasturus* would seem to indicate that there is comparatively little posterior movement of the reproductive organs with the emergence of the subimago in this genus.

Although Spieth (1933) mentioned the close relationship between *Hexagenia* and *Ephemera*, it is noteworthy that *Hexagenia* has penial muscles and *Ephemera* does not.

The genera used in this study may be divided into three groups:

1. Those that have penial muscles having an attachment outside the penis: *Siphonurus*.
2. Those that have intrinsic penial muscles, *i.e.*, muscles that have all their attachments within the penes: *Hexagenia*, *Isonychia*, *Heptagenia*, and *Stenonema*.
3. Those that do not have penial muscles: *Ephemera* and *Blasturus*.

The existence of muscles in the penes of *Siphonurus* which have one of their attachments on the ventrolateral wall of the ninth segment introduces a question as to the embryological origin of these muscles which is beyond the scope of this paper.

Spieth (1933) concluded that *Siphonurus* is the most primitive genus of the 25 genera that he studied, which includes all of the genera used here. It is possible that the type of penial muscles found in *Siphonurus* represents the primitive condition, that those found in *Hexagenia* represent more specialized and advanced conditions, and that the lack of penial muscles, such as seen in *Ephemera*, indicate a different evolutionary branch.

The peculiarity of the posterior attachments of the styliger plate muscles in *Blasturus* indicates that one of two things has happened to the basal segment of the stylus. Either they have disappeared entirely, or they have fused with the styliger plate. From the unusual position of the styli in the genus, the latter would seem to be the case, although there does not appear to be any other evidence to support this.

No positive evidence has been presented here as to the embryological origin of either of the two types of penial muscles. It has been suggested that apparently undifferentiated cells seen in the developing penes of immature nymphs of those species having intrinsic penial muscles may give rise to these muscles. It would probably be necessary to rear large numbers of eggs of one or more of these species in the laboratory, fixing and preserving several individuals at regular intervals from the time that the penes first begin to develop until the last nymphal instar. These would have to be sectioned and carefully studied to establish the embryology of the penial muscles. The same procedure could be followed in studying the penial muscles of *Siphonurus*.

Needham *et al.* (1935) stated that the ejaculatory ducts have a layer of circular muscles enclosing a more or less glandular epithelium. Quadri (1940) claimed that the ejaculatory ducts do not have any circular muscles. Based on the seven species studied, it has been found that the ejaculatory ducts do have a circular muscle coat, but that it is restricted to the basal or anterior half of the ducts in almost all cases. Such muscles are extremely well developed in *H. limbata occulta* and moderately developed in the other species.

The designation of the parts of the genital tube used here is that of Needham *et al.* (1935), except that the whole length of the genital tube that is swollen with spermatozoa is recognized as the seminal vesicle, and not divided, as indicated by Needham, into seminal vesicle and "coiled part of the ejaculatory duct." In all species studied, the terminus of the seminal vesicle and the beginning of the ejaculatory duct was fairly well indicated by an increase in the amount of circular muscle tissue.

SUMMARY

The male reproductive systems of seven species of mayflies were studied by means of reconstructions from serial sections, whole mounts, and microdissections.

Muscles have been found in the penes of mature male nymphs and adults of *Hexagenia limbata occulta*, *Isonychia bicolor*, *Heptagenia hebe*, and *Stenonema rubromaculatum* which are attached only within the penes, having no attachment at the ninth sternite nor at any other part of the insect.

Muscles have been found in the penes of mature male nymphs and adults of *Siphonurus quebecensis* which originate on the ventrolateral walls of the ninth sternite and attach to the dorso-lateral walls of the penes.

The absence of muscles in the penes of mature male nymphs and adults of *Ephemera guttulata* and *Blasturus cupidus* has been noted.

The later development, morphology, and relative disposition of the various parts of the male reproductive systems of some mayflies have been described.

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PLATE III

Figures 1-7. Dorsal aspects of dissections of the posterior portions of the abdomens of male Ephemeroidea imagoes. On each specimen, the styliiger plate and the tenth abdominal segment have been removed. Muscles are shown on the left, the genital tube on the right.

- Figure 1. *Hexagenia limbata occulta*
 Figure 2. *Isonychia bicolor*
 Figure 3. *Siphonurus quebecensis*
 Figure 4. *Stenonema rubromaculatum*

Key:

- | | |
|---------------------------|------------------------|
| ej.d.,—ejaculatory duct. | p. msc.,—penial muscle |
| p.,—penis. | epi.,—epicuticle. |
| s.v.,—seminal vesicle. | exo.,—exocuticle. |
| st.,—stylus. | hyp.,—hypodermis. |
| st. msc.,—stylus muscles. | sp.,—spermatozoa. |

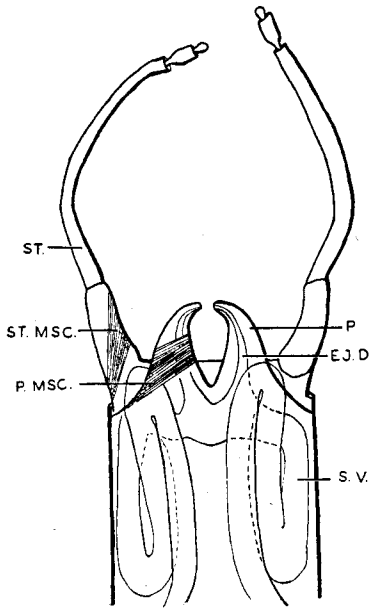


FIG. 1

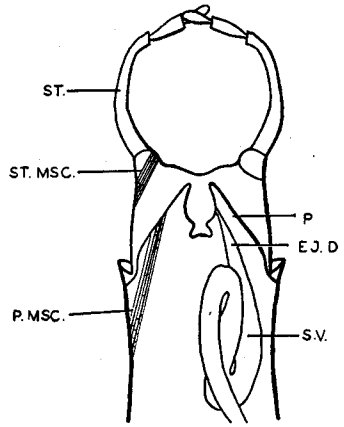


FIG. 3

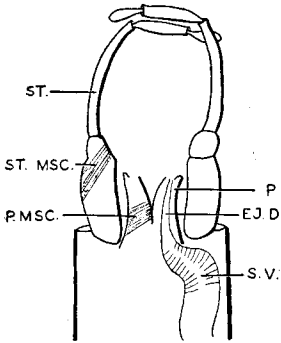


FIG. 2

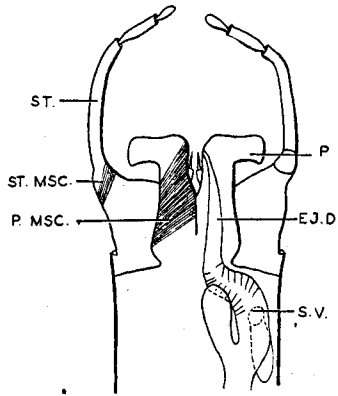


FIG. 4

PLATE IV

- Figure 5. *Heptagenia hebe*
Figure 6. *Ephemera guttulata*
Figure 7. *Blasturus cupidus*
Figure 8. Transverse section through the basal portion of a penis of an
H. limbata occulta imago.

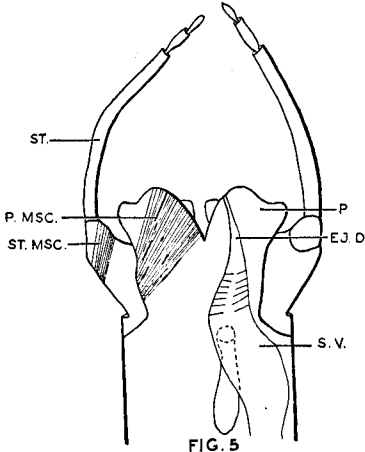


FIG. 5

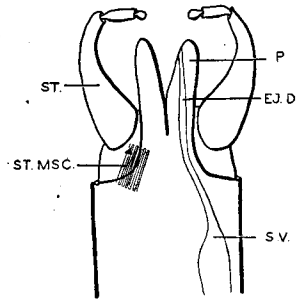


FIG. 7

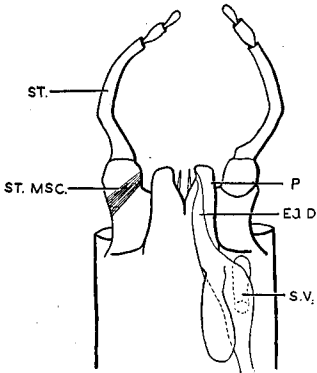


FIG. 6

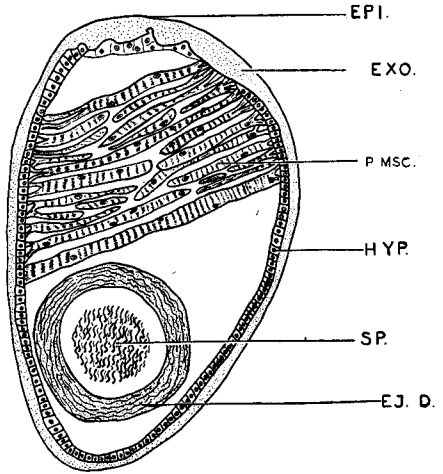


FIG. 8