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The Ultrastructure of the Spermatozoon of *Haplotaxis ornamentus* (Annelida, Oligochaeta, Haplotaxidae) and Its Phylogenetic Significance

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Summary. The spermatozoon of *Haplotaxis ornamentus* has characteristics common to all oligochaete sperm: filiform; primary acrosome vesicle carried on an acrosome tube and containing an axial rod (perforatorium) in an invagination (subvesicular space or secondary acrosomal invagination); an elongate, highly condensed cylindrical nucleus followed by a cylindrical midpiece of radially adpressed mitochondria not penetrated by the axoneme; a single (distal) centriole persistent, though modified, at maturity; axoneme with 9 doublets, each with two outer glycogen granules, and centrally two singlets accompanied by two solid fibres. A peculiar haplotaxid combination of characters (none unique) is slight withdrawal of the primary vesicle into the acrosome tube with a strongly emergent capitulate axial rod and moderately short midpiece. This ultrastructure is consistent with location of the Haplotaxidae at the base of the Haplotaxida (Haplotaxina – Alluroidina – Moniligastrina – Lumbricina). Tubificida sperm, although also plesiomorph for the Oligochaeta, have the autapomorphy elongate periaxial sheath (secondary tube), excepting the Phreodrilidae whose sperm show convergent resemblances to the Lumbricina. The term annuloid has been introduced for annulus-like structures of varied origins.

A. Introduction

The ultrastructure of the spermatozoon has previously been described for one or more species of each of the oligochaete families Lumbriculidae (Order Lumbriculida), Tubificidae, Phreodrilidae and Enchytraeidae (Order Tubificida), Lumbricidae, Megascolecidae and, incompletely, Glossoscolecidae (Order Haplotaxida) (references in Jamieson, 1981b; Ferraguti, 1982). In addition, the spermatozoon of *Sparganophilus tamesis* (Sparganophilidae, Haplotaxida) was described ultrastructurally by Jamieson et al. (1982) and by Richards et al. (1981). Previous knowledge for that of *Phreodrilus* was augmented by Jamieson (1981c) and for the Lumbricidae (*Allolobophora*

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sp. and *Lumbricus terrestris*) by Troyer and Cameron (1980) and Troyer (1980). The majority of oligochaete families remained, and have still to be, investigated for spermatozoal ultrastructure. Of these remaining families none occupies a more significant position for phylogeny and taxonomy than the Haplotaxidae.

In a phylogenetic analysis of opisthoporous oligochaetes and the Haplotaxidae, employing the principles of Hennig (1966), from which spermatozoal characters were excluded, the Haplotaxidae were shown to be the most plesiomorph family of the assemblage (Jamieson 1978a, 1980). In this analysis and in a discussion of the historical biogeography of oligochaetes (Jamieson 1981a) it was suggested that a proto-oligochaete gave rise to two sister-groups, the order Lumbriculida on the one hand and the combined orders Tubificida and Haplotaxida on the other (coordinate ranking of the three taxa was upheld for taxonomic convenience). In the Haplotaxida, the suborders Alluroidina and Moniligastrina have yet to be investigated for spermatozoal ultrastructure but knowledge of this for the Lumbricina (investigated families Sparganophilidae, Glossoscolecidae, Lumbricidae and Megascolecidae) is sufficient to allow us to test in the present study the validity, so far as is deducible from spermatozoal ultrastructure, of regarding the Haplotaxidae as the basal family of the Haplotaxina – Alluroidina – Monilgastrina – Lumbricina assemblage. It is also proposed to make some comments on the phylogenetic position of the Tubificida relative to the Haplotaxidae. The Haplotaxidae were regarded by Brinkhurst and Fulton (1980) and Brinkhurst (1981) as ancestral not only to the remaining Haplotaxida but also to the Tubificida. (The disagreement is largely one of terminology: we are in accord that the Tubificida (or Tubificina) and Haplotaxida (or Haplotaxina) descend from a common, at least octogonadial ancestor but I consider, contrary to Brinkhurst, that this ancestor would not strictly be placeable in the Haplotaxidae). The supposedly basal position of the Lumbriculidae in the Oligochaeta will also be briefly discussed.

The description of the sperm of *Haplotaxis* will not be as complete as desired owing to some inadequacies of fixation resulting from preservation of the material in glutaraldehyde for ten days at ambient, often high, temperatures but the pivotal position of haplotaxids in phylogenetic studies warrants this report. The extended fixation was necessitated by difficulties in collecting, fixing and dispatching *H. ornamentus* from Great Lake, Tasmania, tasks kindly undertaken by Mr. Wayne Fulton, to whom the author is deeply indebted.

B. Material and Methods

Portions of *H. ornamentus* Brinkhurst & Fulton, 1980, containing the reproductive organs were fixed in 3 percent glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, for 1 day at 4° C and for 9 days at ambient temperatures; rinsed in the same buffer; post-fixed in similarly buffered 1 percent osmium tetroxide solution for 80 min; rinsed in the buffer; dehydrated in a graded ethanol series (at 4° C from 20 through 70 percent, thereafter 20° C); and embedded in Spurr's low viscocity epoxy-resin. Seminal vesicles and funnels were dissected out and thin sections of these were cut on an LKB 2128 UM IV ultrotome and mounted

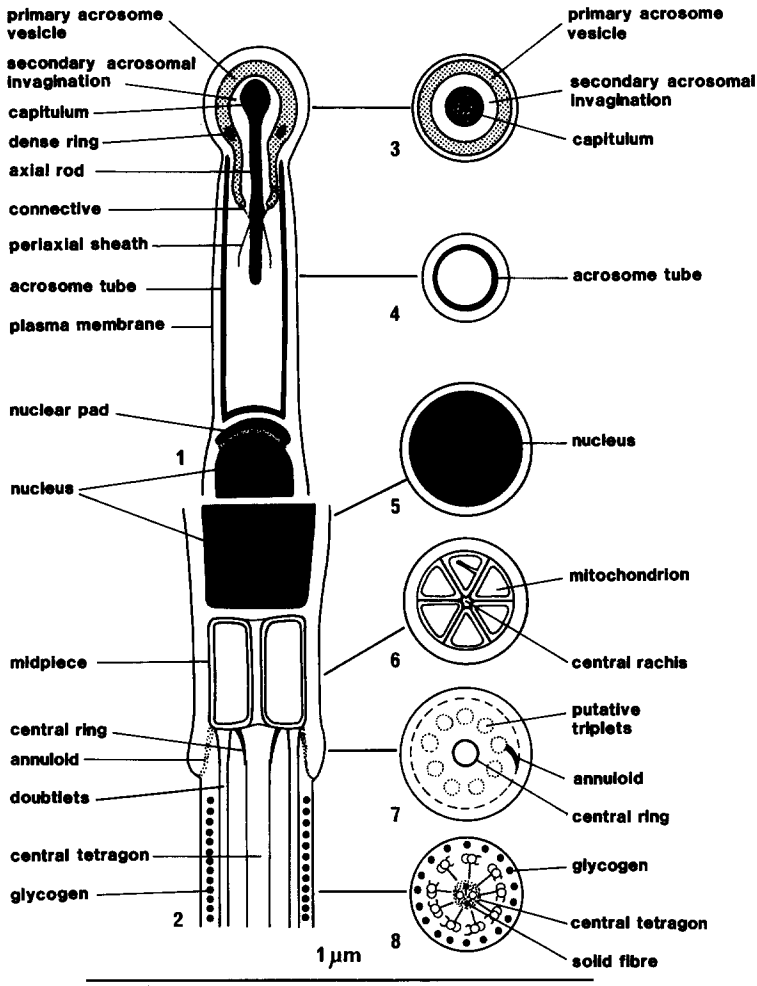
on formvar-coated H200 copper grids. Sections were stained with 5 percent aqueous uranyl acetate for 40 min and Reynold's lead citrate for 20 min. Micrographs were taken on AEI Corinth and Phillips 400 electron microscopes at 60–80 kv.

C. Results

Unless otherwise stated, observations are of mature spermatozoa on the seminal funnels. To avoid repetition in the discussion, some comparison of the haplotaxid spermatozoon with that of other families will be made in this section. In the account, "proximal" and "distal" refer to the acrosomal and tail ends of the spermatozoon respectively. The proximal end is that attached, in the spermatid, to the cytophore.

a) The Acrosome

Basic oligochaete features of the haplotaxid acrosome (Figs. 1, 3, 4, 9–17) are the acrosome tube (seen also in the Hirudinea) capped by the concavo-convex primary acrosome vesicle. As in the Tubificidae, but unlike the Enchytraeidae, the posterior rim of the acrosome vesicle is withdrawn for a short distance (considerably less than half its length) into the proximal end of the acrosome tube (Figs. 1, 10). The larger, emergent portion of the vesicle and its investing plasma membrane form a large bulbous termination to the acrosome. A concentration of dense material (Figs. 1, 13) within the vesicle, proximal to the acrosome tube, constitutes the dense ring also known for *Lumbricus* (Anderson and Ellis 1968) and the tubificid *Limnodriloides winckelmanni* (Jamieson and Daddow 1979). Subacrosomal material is present in the form of the longitudinal axial rod which occupies the space (subacrosomal space or secondary acrosomal invagination, a term preferable to "secondary acrosome vesicle" of Jamieson 1981 b) surrounded by the inner wall of the primary vesicle; the rod projects for a short distance distally of this (Figs. 1, 10–12). Like the investigated opisthopores (Sparganophilidae, Lumbricidae and Megascolecidae) the proximal end of the rod is dilated and is here identified with the megascolecid capitulum (Jamieson 1978 b). As in the Enchytraeidae, Tubificidae and Sparganophilidae, the axial rod lies at the proximal end of the acrosome tube. This contrasts with the situation in phreodrilids, lumbricids and megascolecids in which the distal end of the rod withdraws during acrosome morphogenesis far below the proximal end of the tube, in the lumbricids and megascolecids lying within the distal half of the tube. In *Haplotaxis,* however, the anterior end of the axial rod projects well proximal of the tube, a condition known only in the Enchytraeidae and Sparganophilidae. In *Sparganophilus* projection, though considerable, involves only a small fraction of the rod; whereas in enchytraeids, like *Haplotaxis,* approximately half of the rod is external to the tube. A periaxial sheath (secondary tube), so well developed in the Tubificida, and dependent from the posterior rim of the primary acrosome vesicle, is moderately developed, though rarely visualized (Fig. 11), and apparently does not extend posterior to the axial rod. Its



Figs. 1–8. *Haplotaxis ornamentus.* Drawings of the mature spermatozoon from the seminal funnel prepared from tracings of micrographs

Fig. 1. Longitudinal section of the acrosome on the tip of the nucleus. The existence of connectives from the rim of the primary vesicle requires confirmation

Fig. 2. Nucleus (distal end), midpiece and proximal region of axoneme

Figs. 3–8. Transverse sections of the regions indicated on the longitudinal sections. In Fig. 7, the centriolar equivalent, only one density of the annuloid (*broken line*) is indicated and microtubules of the putative triplets have not been satisfactorily resolved. In Fig. 8, the doublets were poorly resolved and electron dense but doublet structure and diagrammatically indicated dynein arms were demonstrated

anterior portion appears to form short connectives from the vesicle to the rod (Figs. 10, 11).

Distal to the rod in the haplotaxid acrosome tube, there is a very long basal chamber consisting solely of the walls of the tube and lacking internal components (Figs. 1, 4, 10–12, 17). This resembles the condition in *Phreodrilus* and *Sparganophilus* and contrasts with enchytraeids and tubificids in which, despite proximal projection of the rod, the distal end of the rod is not far removed from the nucleus. The basal chamber of lumbricid and megascolecid acrosomes is also short owing to deep withdrawal of the primary vesicle and the enclosed rod.

The junction between the acrosome and the domed tip of the nucleus is formed by a thick, strongly electron dense concavo-convex pad which is attached to the nucleus by less dense material (Figs. 1, 12, 14). There is a suggestion of this in enchytraeids and tubificids; its presence is uncertain in phreodrilids; it is moderately developed in lumbricids and well developed in sparganophilids and megascolecids.

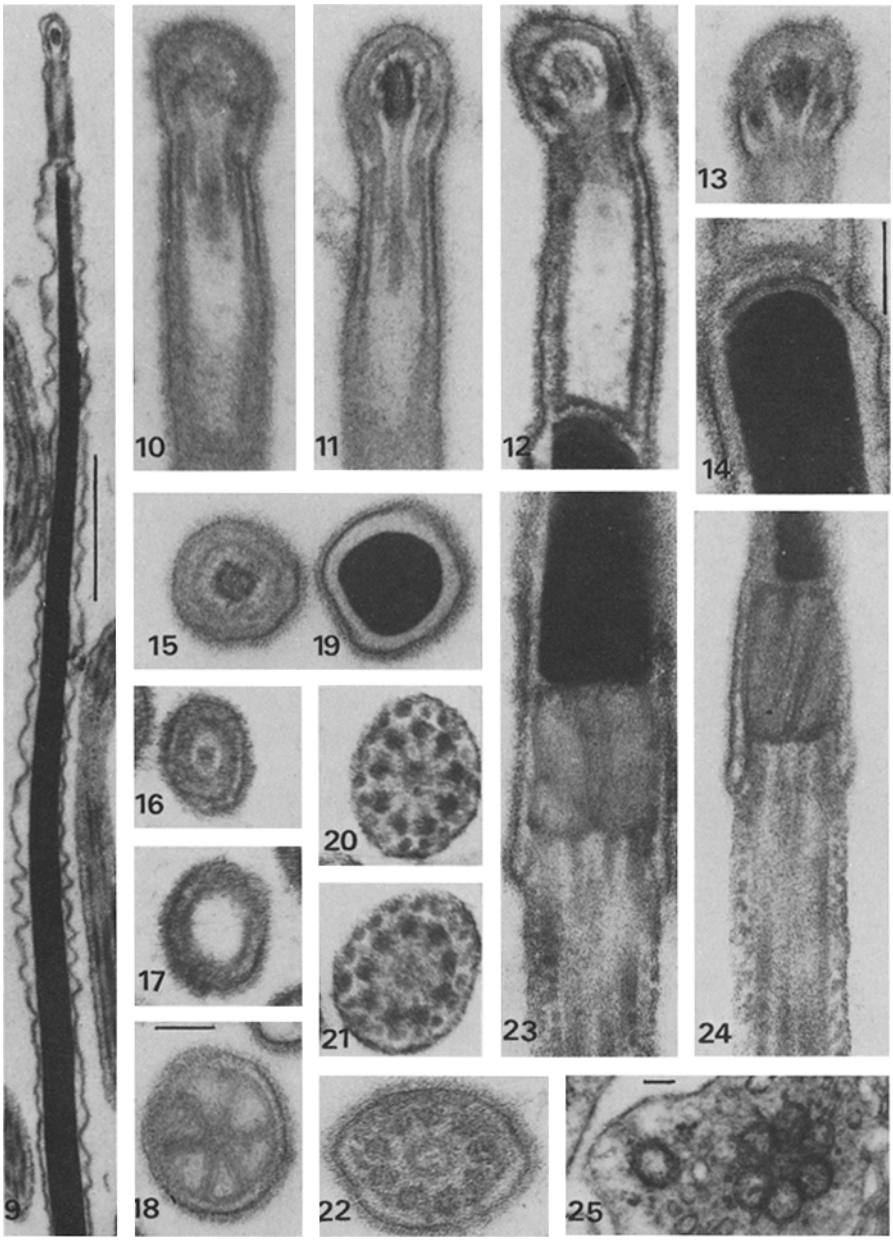
The acrosome tube is open basally in all investigated families excepting, apparently, the Haplotaxidae. In this family the broad base is spanned by an uninterrupted continuation of the walls. It seems unlikely, from the considerable number of apparently axial longitudinal sections which have been investigated, that a median perforation of the base of the tube would have been missed. Nor has a perforation been detected in transverse sections through the base of the acrosome tube. No projecting boss from the underlying nuclear pad, which in *Sparganophilus* penetrates the central perforation of the base of the tube, is present. The continuous base to the tube is clearly the homologue of the shelf-like limen which results from such a central opening in the phreodrilid, sparganophilid, megascolecid and (though poorly developed) lumbricid acrosome tube.

b) The Midpiece

In *Haplotaxis* the midpiece, although more elongate than in the Tubificidae, is short in relation to width when compared with lumbriculids, enchytraeids, phreodrilids, lumbricids and megascolecids but resembles that of *Sparganophilus*. The length is 0.27–0.39 μm, mean 0.31 μm ($n=3$) and the width (mitochondria only) 0.17–0.23 μm, mean 0.19 μm ($n=4$). The six mitochondria are arranged with their long axes parallel to, or sometimes somewhat oblique to, the central axis. This variable condition constitutes an intermediate between the straight condition of the midpiece mitochondria in all opisthopores and the spiral form in lumbriculids, enchytraeids and phreodrilids. In tubificids it is too short to be capable of coiling.

c) The Centriole and Flagellum

As in all other oligochaete sperm, the proximal centriole does not survive completion of spermiogenesis and the equivalent of the distal centriole becomes located behind the midpiece. As a result a condition which is rare



Figs. 9–25. *Haplotaxis ornamentus.* Transmission electron micrographs of the mature spermatozoon or, in Fig. 25, or a spermatid. Except where a bar-line is included, the scale is as indicated in Fig. 18 (0.1 μm). The bar-line for Fig. 9 indicates 1 μm, for Figs. 14 and 25, 0.1 μm

Fig. 9. Longitudinal section of the proximal end of the spermatozoon showing much of the nucleus and the terminal acrosome with pimary vesicle borne on the acrosome tube

in the Metazoa ensues in which the axoneme does not penetrate the midpiece mitochondria. This condition is seen also in leeches (references in Jamieson 1981 b), Onychophora (Baccetti and Dallai 1977) and Pogonophora (Franzén 1973, 1977).

Fixation is not adequate for full resolution of centriolar and axonemal ultrastructure but it is clear that in *Haplotaxis* the centriolar equivalent differs from that known for other oligochaetes. The chief difference consists in proximal extension of a central ring, which is normally present at the beginning of the oligochaete sperm axoneme, into the centriole (Figs. 7, 22) so as to join the posterior wall of the midpiece (Figs. 2, 23, 24). A similar central ring is known for *Tubifex, Sparganophilus* and (Troyer and Cameron 1980) lumbricids but there it is limited to the commencement of the central singlets whereas the centriole proper, more proximally, lacks structures central to its peripheral triplets. The latter condition resembles that in megascolecids in which, however, the central ring has a solid core. That the peripheral elements of the haplotaxid centriolar equivalent (Figs. 7,

Figs. 10–12. Longitudinal sections of three acrosomes. Withdrawal of the rim of the primary vesicle a short distance into the acrosome tube is seen in Figs. 10 and 11, in which there are suggestions of connectives to the axial rod. Figure 11 demonstrates the periaxial sheath (secondary tube). Figure 12 best exemplifies the acrosome tube, showing the complete, domed transverse base

Fig. 13. Emergent portion of primary vesicle including the dense ring and showing the capitulum in the secondary invagination

Fig. 14. Junction of acrosome and nucleus showing the intervening nuclear pad

Figs. 15–17. Transverse sections of the acrosome; **15**, through the capitulum; **16**, through the axial rod; **17**, through the acrosome tube distal to the axial rod in the long equivalent of the basal chamber of other families

Fig. 18. Transverse section of the midpiece, showing the radiating radial walls of the six mitochondria

Fig. 19. Transverse section of the nucleus.

Figs. 20 and 21. Transverse sections of the chief, glycogen-bearing region of the axoneme. The two solid fibres accompanying the two central singlets in a tetragon arrangement are best seen in Fig. 20

Fig. 22. Transverse section of the centriolar equivalent consisting of 9 putative triplets surrounding the central ring; dense material (apparently discontinuous) peripheral to the triplets is the annuloid

Fig. 23 and 24. Longitudinal section of the midpiece region, showing the annuloid connecting the posterolateral aspect of the mitochondria to the plasma membrane and, attached to the mitochondria centrally, the central ring of the axoneme. Glycogen granules surround the doublets of the axoneme as in Figs. 20 and 21

Fig. 25. Transverse section of the spermatid through the six still rounded mitochondria of the developing midpiece and (*left*) through the acrosome tube, in the vicinity of the Golgi apparatus (*far right*)

22) are triplets has not been certainly resolved but is suggested in Fig. 22. Peripheral to these putative triplets there is a periodically interrupted ring of dense material, here termed the annuloid (see Discussion), partially visible in Fig. 22. In longitudinal sections (Figs. 2, 23, 24) this is seen to extend from the posterolateral extremity of the midpiece mitochondria to the plasma membrane at the level of the posterior limit of the central ring. The plasma membrane covers a node-like widening of the spermatozoon at the level of the annuloid, terminating at the posterior limit of the latter. The annuloid is here considered the homologue of the centriolar satellites known for several oligochaete families.

The axoneme, which is proximally 0.23 μm wide, including the plasma membrane, is characteristic of oligochaete sperm: nine doublets with dynein arms are joined by radial spokes (Afzelius arms) to a central core which consists of two singlet microtubules augmented, as is usually the case in oligochaetes, by a pair of thin solid fibres giving a "tetragon" configuration; putative glycogen granules lie under the plasma membrane, two to each doublet (Figs. 2, 8, 20, 21). The exact configuration of these granules is not well resolved but is reviewed for other taxa in Jamieson (1981b). Although internal projections into the lumina of the doublet microtubules have been resolved in sperm axonemes of other oligochaetes, it is possible that the strong electron density of the doublets in *Haplotaxis* (Figs. 20, 21) is at least partly artefactual. Nevertheless, fixation alone cannot account for this density as cilia in the same preparations show distinct doublet lumina.

D. Discussion

It has been shown above that the spermatozoon of *Haplotaxis ornamentus* conforms to the general oligochaete pattern in the following features. It is filiform with a proximal acrosome which consists of a primary acrosome vesicle invaginated to enclose, in a subvesicular space (secondary acrosomal invagination), a perforatorium-like axial rod, these surmounting an acrosome tube; the nucleus is cylindrical and highly condensed; behind it two to eight (here six) radially adpressed mitochondria form the cylindrical midpiece, the mitochondria of which are more elongate than the round mitochondria of primitive sperm; the midpiece is not penetrated by the axoneme and a proximal centriole is absent from the mature spermatozoon; the axoneme commences with a modified distal centriole; there are nine peripheral axonemal doublets, each associated with two outer glycogen granules, and two central singlets which are accompanied by two slender solid fibres.

Although conforming to these generalizations, the haplotaxid sperm confirms the statement that "each oligochaete family has its own distinctive spermatozoal ultrastructure (with intrafamilial variation) and that the degree of modification of the acrosome broadly corresponds with the phylogenetic positions deduced on other grounds for the major groups" (Jamieson et al. 1982). Thus, the combination of characters described in the Results for the haplotaxid sperm is distinctive although to a considerable extent

a matter of relative proportions of common components. No other spermatozoon combines slight withdrawal of the primary acrosome vesicle into the tube with a strongly emergent capitulate axial rod and a moderately short midpiece. Connection of the central ring of the axoneme (via the centriolar equivalent) with the midpiece may be unique.

However, strong projection of a capitulate axial rod beyond the acrosome tube, and the relatively short midpiece, are characteristic of the Sparganophilidae, the next "highest" member of the order Haplotaxida in the Hennigian analysis of Jamieson (1978a, 1980) to have been investigated for spermatozoal ultrastructure. In *Sparganophilus,* the primary acrosome vesicle, although retaining as in *Haplotaxis* a large external bulb, has, appropriately to its phylogenetic position, withdrawn more deeply into the acrosome tube. Thus the ultrastructure of the spermatozoon of *Haplotaxis* is remarkably close to what would be envisaged for a family arising from the base of the alluroidin-moniligastrin-lumbricin assemblage with which it constitutes the Haplotaxida. We may allow ourselves the prediction that alluroidid and moniligastrid spermatozoal ultrastructure will prove to be intermediate between that of *Haplotaxis* and *Sparganophilus* if they show no major departures in fertilization biology.

The relative similarity of the haplotaxid sperm with that of *Sparganophilus,* while appearing to confirm the phylogenetic value and conservatism of spermatozoal ultrastructure, is nevertheless remarkable in view of an important difference in reproductive biology: the eggs of the Lumbricina, including *Sparganophilus,* are small with only moderate amounts of yolk, whereas those of all other oligochaetes, from the Moniligastridae down to the Lumbriculidae, are large and yolky, the two conditions correlating with a multilayered and a single layered clitellum, respectively. It would be surprising if no difference in spermatozoal ultrastructure could be related to these differences and it is, perhaps, relevant in this regard that the length of the haplotaxid acrosome, at 1μm or less, conforms with that for lumbriculids, enchytraeids and tubificids while that of *Sparganophilus,* at 1.5 μm, corresponds with the lower end of the known range for megadriles which is 1.7 μm for *Amynthas* in contrast with more than 7 μm for *Lumbricus.*

It is, perhaps, too early to attempt to use spermatozoal ultrastructure to decide between the alternatives that the Tubificida should be included (as a suborder) within the Haplotaxida (Brinkhurst and Jamieson 1971; Brinkhurst and Fulton 1980; Brinkhurst 1981) or should be excluded as the sister-group of the Haplotaxida (Jamieson 1978a, 1980, 1981a; Timm 1981). The evidence, however, is more consistent with the latter relationship.

We may envisage a common ancestor of the Lumbriculida, Tubificida and Haplotaxida with an acrosome like that of the Enchytraeidae in having a short acrosome tube, wholly external primary vesicle and strongly emergent, simple axial rod but differing from the enchytraeids in having only a rudimentary periaxial sheath (secondary tube) dependent into the tube from the posterior rim of the primary vesicle; the nucleus probably lacked helical structure and the midpiece would have been short. From this arose the Lumbriculida (though their position is debatable) whose sperm are im-

perfectly known but which developed an helical form. Their presumed sister-taxon gave rise to the Tubificida and the Haplotaxida. In the Tubificida the enchytraeids acquired an helically flanged nucleus and spiral midpiece but retained an acrosome little changed from the presumed proto-oligochaete condition beyond elongation of the periaxial sheath; in the Tubificidae withdrawal of the primary acrosome vesicle into the longer acrosome tube commenced, the axial rod penetrated deeply into the tube and was scarcely emergent, and the periaxial sheath greatly elongated. *Phreodrilus* sp., aberrant in its biology in being epizoic on crayfish (free living forms have not been examined for sperm ultrastructure) convergently acquired spermatozoal characteristics of the lower Lumbricina such as deep withdrawal of the rim of the primary acrosome vesicle, with the axial rod, into the acrosome tube and non-elongation of the periaxial sheath and vied with the Megascolecidae in elongation of the acrosome tube though this did not attain the lengths seen in the Lumbricidae. It will be of the greatest interest to examine the fertilization biology of phreodrilids for departure from the normal tubificid mode which might explain lumbricid-like features of the acrosome. Affinity with other Tubificida is, nevertheless, retained in the absence of a capitulum to the axial rod.

The Haplotaxida are united in withdrawal of the primary vesicle into an elongating acrosome tube, a withdrawal which shows a progression remarkably consistent with the previously proposed Hennigian phylogeny, development of a capitulate acrosome rod which is still emergent in the lower members (Haplotaxidae, Sparganophilidae) and failure of the periaxial sheath to elongate to the extent seen in Tubificidae while it becomes involved in formation of connectives to the rod which are also seen in *Phreodrilus* and Lumbricina.

The term annuloid has been introduced in the present work for the dense material which lies peripherally to the centriolar equivalent (basal body) in the spermatozoon of *Haplotaxis* and which connects posteriorly to the plasma membrane and anteriorly to the posterolateral aspect of the mitochondria of the midpiece. This material is seen in cross section to consist of a discontinuous ring of discrete densities. There is little doubt that it is a persistence of the nine satellites which, with radial rays connecting them to the centriolar triplets, make up the satellite complex which has been clearly demonstrated in the spermatid (*Haplotaxis,* Jamieson unpublished; *Lumbricus terrestris,* Anderson et al. 1967; *Tubifex tubifex,* Ferraguti and Lanzavecchia 1971; megascolecids, Jamieson 1978b; *Allolobophora* sp., Troyer and Cameron, 1980).

It appears that the dense ring or collar described by Anderson et al. (1967) for the spermatozoon of *Lumbricus terrestris* and by Jamieson and Daddow (1979) for *Limnodriloides winckelmanni* is identical with the annuloid of *Haplotaxis*. Suggestions that in *Lumbricus* the ring is discrete from the satellite complex and is uninterrupted do not appear to be substantiated.

The value of the term annuloid lies in its applicability to known satellite complexes, whether modified or not, and to undetermined densities peripheral to the basal body irrespective of whether they are subsequently translo-

cated. The annuloid may on more profound examination prove to be a true annulus, a modified or normal satellite complex or a distinct structure. Use of the term will obviate incorrect or premature attribution of peripheral densities to one or other of these categories. The need for the term may be exemplified by use of the terms ring centriole, annulus or dense ring synonymously, for a structure said to resemble a satellite complex, in the vicinity of the basal body of the spermatid of the gastropod *Littorina sitkana* by Buckland-Nicks and Chia (1976); and of annulus, accessory centriole or ring centriole for a 9-rayed structure in the spermatid of the polychaete *Polydora ligni* by Rice (1981). A dense ring in the advanced spermatid of *Polydora ciliata* has also been described as "suggestive of an annulus" by Franzén (1974). That these structures are homologous with the mammalian annulus (also to be included under the term annuloid) is questionable.

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