

FINE STRUCTURE OF SPERM TAILS OF ISOPODS

BY

ROBERTA E. BLANCHARD¹⁾, RALPH A. LEWIN²⁾, and DELBERT E. PHILPOTT³⁾

Marine Biological Laboratory, Woods Hole, Massachusetts

INTRODUCTION

Electron microscopy has revealed a remarkable uniformity in the fine structure of motile spermatozoa from animals as diverse as protozoa, molluscs, echinoderms and vertebrates. Sperm tails in most phyla, like other flagella and cilia of both plants and animals, consist essentially of a cylinder of nine double fibrils enclosing a central pair, the axis so formed being embedded in a structureless matrix and bounded by a limiting membrane (Bradfield, 1955). Among the Crustacea, however, certain anomalous types have been found (see Discussion). In view of the apparent lack of motility of isopod sperm tails, and their comparative length and resiliency, it was considered of interest to examine representative specimens under the electron microscope. Three species were investigated by this means: *Idotea balthica* (Pallas) (marine), *Cyathura* sp. (from brackish water) and *Oniscus asellus* L. (terrestrial). Several specimens of *Asellus militaris* (Hay) (a fresh-water species) were examined, but the vasa deferentia were found to contain only apparently immature sperm, without tails. In addition, tailed sperm of the fresh-water amphipod *Gammarus fasciatus* Say were examined by the light microscope, but material embedded and sectioned proved unsatisfactory for electron microscopy.

METHODS

Whole spermatozoa from vasa deferentia of *Cyathura* were fixed in 10% formalin, washed with distilled water, allowed to dry on a formvar membrane supported on a fine-mesh grid, and directly examined under the electron microscope (RCA model EMU-2).

Vasa deferentia containing ripe sperm were dissected from adult males, fixed with 1% osmium tetroxide in veronal buffer and isotonic saline, washed, dehydrated in a propanol series, and imbedded in a 3 : 1 mixture of butyl and methyl methacrylate. Thin transverse and longitudinal sections were cut with a special microtome (Philpott, 1955) and examined under the electron microscope.

OBSERVATIONS

In each case the sperm tails are long (up to 1 mm in *Cyathura*), cylindrical,

¹⁾ Present address: Woods Hole Oceanographic Institution, Woods Hole, Mass., U.S.A.

²⁾ Present address: Scripps Institution of Oceanography, La Jolla, Calif., U.S.A.

³⁾ Present address: Boston University, Boston, Mass., U. S. A.

though tapered distally to a fine point, and resilient. Although kinks were occasionally noted in *Oniscus* and *Gammarus*, motility was not observed in any of the species we examined.

Electron microscopy reveals the structure to consist of a series of discs, seen in longitudinal section as transverse bands or striations of various thicknesses and electron densities, with a regularly repeating periodicity. A segmented central core 40-50 $m\mu$ wide, with denser walls and a less dense medulla, is evident in median longitudinal and in transverse sections. A summary of measurements is given in Table I.

TABLE I
Axial and band widths ($\pm 2 m\mu$) of isopod sperm tails.

	<i>Cyathura</i>	<i>Idotea</i>	<i>Oniscus</i>
	$m\mu$	$m\mu$	$m\mu$
Diameter of tail axis	300	400	240
Repeating period	63	57	62

The whole mount of *Cyathura* sperm (fig. 1) shows the tail axis enclosed in a sheath of less dense material, which exhibits transverse bands corresponding to those of the axis itself. In the course of drying, this sheath presumably flattened out to appear wider than in life. The longitudinal sections (fig. 2) show that each repeating period consists of four bands, with the following approximate widths: dark 18 $m\mu$, intermediate density 16 $m\mu$, pale 12 $m\mu$, intermediate 16 $m\mu$. The pale central medulla tends to be interrupted in the region of the darkest bands, and to widen in the proximal region near the head (figs. 3-6).

In *Idotea* (figs. 7, 8) each repeating period also appears to consist of four bands: pale 16 $m\mu$, dark 8 $m\mu$, intermediate 20 $m\mu$, dark 8 $m\mu$. A thin, pale streak is occasionally visible in the middle of the intermediate band. It would be premature to attempt to homologize this band pattern with that of *Cyathura*. The cross sections, however, are similar.

In *Oniscus* (fig. 9) each period comprises three faint and one darker band about 8 $m\mu$ wide, equally spaced along a pale background. Since our preparations of this species were less satisfactory than the others, these details were unclear. Cross sections resemble those of the other two isopods; but here they show the sperm associated in bundles of about ten (Retzius, 1909; Vandel, 1934).

DISCUSSION

The spermatozoa of crustaceans include a variety of unusual and bizarre forms. Though published data are scattered and fragmentary, the following generalizations may be made (cf. Retzius, 1909):

1. Motile spermatozoa of unique construction and extreme length (as long as 10-20 mm!) occur in the sub-class Ostracoda (Lowndes, 1935). Those of *Notodromas* have been examined with the electron microscope (Bradfield, 1955).

2. Spermatozoa with tails and active motility occur in the sub-classes Branchiura and Cirripedia. Preliminary electron microscope studies of barnacle sperm (Dr. H. Barnes, personal communication) indicate that they consist of fibrils similar to those of other animals.

3. Tail-less spermatozoa occur in the sub-classes Branchiopoda and Copepoda.

4. Among the Eumalacostraca, tail-less sperm occur in the Eucarida (euphausids and decapods³) and the Hoplocarida (stomatopods), and tailed sperm in the Peracarida (isopods, amphipods, mysids and cumaceans). In the latter division, however, the tails are wholly unlike those of sperm from other animals. They are relatively long (often exceeding 1 mm), resilient or pliant, and normally non-motile.

Retzius (1909) reported that he had been unable to observe movement in the sperm of any of the Peracarida examined, and this has been the general experience (e.g. in *Trichoniscus*; Vandel, 1934). We likewise observed no movement in four species of isopod, nor in the amphipod *Gammarus*. Among the amphipods, however, there is a record of worm-like movement of the heads of caprellid sperm (Reibisch, 1926, p. 782), and of a slow vermiform movement in the tails of *Talitrus* sperm when in close proximity to eggs removed shortly after their discharge into the brood pouch (Williamson, 1951). Since Lowndes (1935) observed motility of ostracod sperm only within the spermathecae of female animals, it is possible that isopod and other crustacean sperm may be found to be motile if suitable physiological conditions are provided. In isopods the sperm tails may play no direct role in propulsion, but may serve as "handles" to facilitate the mechanical transfer of sperm bundles by the specially modified 2nd pleopods of the male. In this connection it should be mentioned that, even in mammals, the autonomous motility of spermatozoa may play a less important role in their translocation than is generally supposed (Hartmann, 1957).

The extreme conservatism of sperm tail construction in other classes of the animal kingdom suggests that the fine structure of crustacean sperm tails may provide valuable clues to phylogenetic affinities. The sperm tails in other groups of the Peracarida, and in the Syncarida, might prove of special interest in this connection.

A regular periodicity of light and dark bands is characteristic of collagen fibrils, with a periodicity of 64 μ (see review by Banfield, 1958), and of paracrystals of certain fibrous proteins such as paramyosin, with a period of 72 μ (Hall *et al.*, 1945). Of perhaps more immediate pertinence is the transverse striation of the intracellular rootlets of cilia in many organisms (e.g. annelids, molluscs), where the repeating period of 66-70 μ is subdivisible into several bands, like that of collagen (Banfield, 1958). In particular, the single ciliary rootlets of the coelen-

³ The sperm cells of penaeids bear a short, fine process, 3 to 5 μ long (Heldt, 1938; Hudinaga, 1942). In two species, Heldt even reported a slow, trembling rotation of the sperm, but this may possibly have been Brownian movement.

terates *Mussa* (period, 67 μ ; Goreau & Philpott, 1956) and *Metridium* (period, 52 μ ; Grimstone *et al.*, 1958) appear to be organically continuous with the basal granule of the cilium, and to show indications of a less dense central core. Thus in some respects they resemble the sperm tails of isopods described above, though the periodic bands have not been resolved further into distinguishable subdivisions. Since evidence from a variety of recent studies suggests a homology between basal granules and centrioles, it is significant that the sperm tails of *Asellus nipponensis*, like those from animals of other phyla, apparently arise from one of the centrioles (Sugiyama, 1933).

Transverse striation of the isopod sperm tail also recalls the fine structure of the stalks of *Zoothamnium*, a colonial ciliate, in which cilia of the classical construction, with 9 + 2 fibrils, apparently undergo transition into a hollow, transversely striated tube with a periodicity of 44-47 μ (Rouiller *et al.*, 1956). Collagen-like fibrils with a similar banding have rarely been found in arthropods, though there are a few such records (e.g. Gray, 1959). One should not speculate further on possible homologies until the chemical nature of the sperm tails of isopods and allied crustaceans has been established.

Acknowledgements

The authors are grateful to Dr. W. D. Burbanck for having brought the immotility of *Cyathura* sperm to their attention, and for providing animals of this genus.

This work was supported by the National Institutes of Health under Research Grant E. 1445.

RÉSUMÉ

La microscopie électronique a révélé un type unique de flagelle caudal chez les spermatozoïdes des Isopodes *Cyathura* sp., *Idotea balthica* et *Oniscus asellus*. Ce flagelle consiste en un long cylindre flexible (ou élastique), de 300-350 μ de diamètre, transversalement strié à travers toute son épaisseur avec une alternance régulière de bandes denses et de bandes moins denses. Il y a une partie axiale segmentée, mais aucune preuve d'une structure fibrillaire analogue à celle que l'on a trouvée dans toutes les queues de spermatozoïdes examinées jusqu'ici avec le microscope électronique. Les implications phylogénétiques de cette structure inhabituelle sont discutées.

LITERATURE CITED

- BANFIELD, W. G., 1958. Collagen and reticulon. In: *Frontiers in Cytology*, pp. 504-519. Ed. S.L. Palay, Yale Univ. Press, New Haven.
- BRADFIELD, J. R. G., 1955. Fibre patterns in animal flagella and cilia. *Symp. Soc. exper. Biol.*, **9**: 306-334.
- GOREAU, T. F., and D. E. PHILPOTT, 1956. Electronmicrographic study of flagellated epithelia in madreporarian corals. *Exper. Cell Res.*, **10**: 552-556.
- GRAY, E. G., 1959. Electron microscopy of collagen-like connective tissue fibrils of an insect. *Proc. R. Soc. (B)*, **150**: 233-239.
- GRIMSTONE, A. V., R. W. HORNE, C. F. A. PANTIN, and E. A. ROBSON, 1958. The fine structure of the mesenteries of the sea-anemone, *Metridium senile*. *Q. Journ. microscop. Sci.*, **99**: 523-540.
- HALL, C. E., M. A. JAKUS, and F. O. SCHMITT, 1945. The structure of certain muscle fibrils as revealed by the use of electron stains. *J. appl. Phys.*, **16**: 459-465.
- HARTMAN, C. G., 1957. How do sperms get into the uterus? *Fert. and Ster.*, **8**: 403-427.

- HELDT, J. H., 1938. La reproduction chez les crustacés décapodes de la famille des Pénéides. Ann. Inst. Océanogr., **18**: 31-206.
- HUDINAGA, M., 1942. Reproduction, development and rearing of *Penaeus japonicus* Bate. Jap. Journ. Zool., **10**: 305-393.
- LOWNDES, A. G., 1935. The sperms of freshwater ostracods. Proc. zool. Soc. Lond., 1935 (1): 35-48.
- PHILPOTT, D. E., 1955. A simple and economical microtome for ultra-thin sectioning. Exper. Med. and Surg., **13**: 189-192.
- REIBISCH, J., 1926-7. Amphipoda. In: Handbuch der Zoologie, pp. 767-808. Ed., W. Kükenthal and T. Krumbach, W. de Gruyter, Berlin and Leipzig.
- RETZIUS, G., 1909. Die Spermien der Crustaceen. Biologische Untersuchungen, N.F. **14**: 1-54.
- ROUILLER, C., E. FAURÉ-FREMIET, and M. GAUCHERY, 1956. Origine ciliaire des fibrilles scléroprotéiques pédonculaires chez les ciliés péritriches. Exper. Cell Res., **11**: 527-541.
- SUGIYAMA, M., 1933. The spermatogenesis of *Asellus nipponensis*. J. Fac. Sci. Tokyo, **3**: 169-176.
- VANDEL, A., 1934. La parthénogénèse géographique. II. Les mâles triploïdes d'origine parthénogénétique de *Trichoniscus* (*Spiloniscus*) *elizabethae* Herold. Bull. Biol., **68**: 419-463.
- WILLIAMSON, D. I., 1951. On the mating and breeding of some semi-terrestrial amphipods. Dove Marine Laboratory, Report **12**: 49-61.

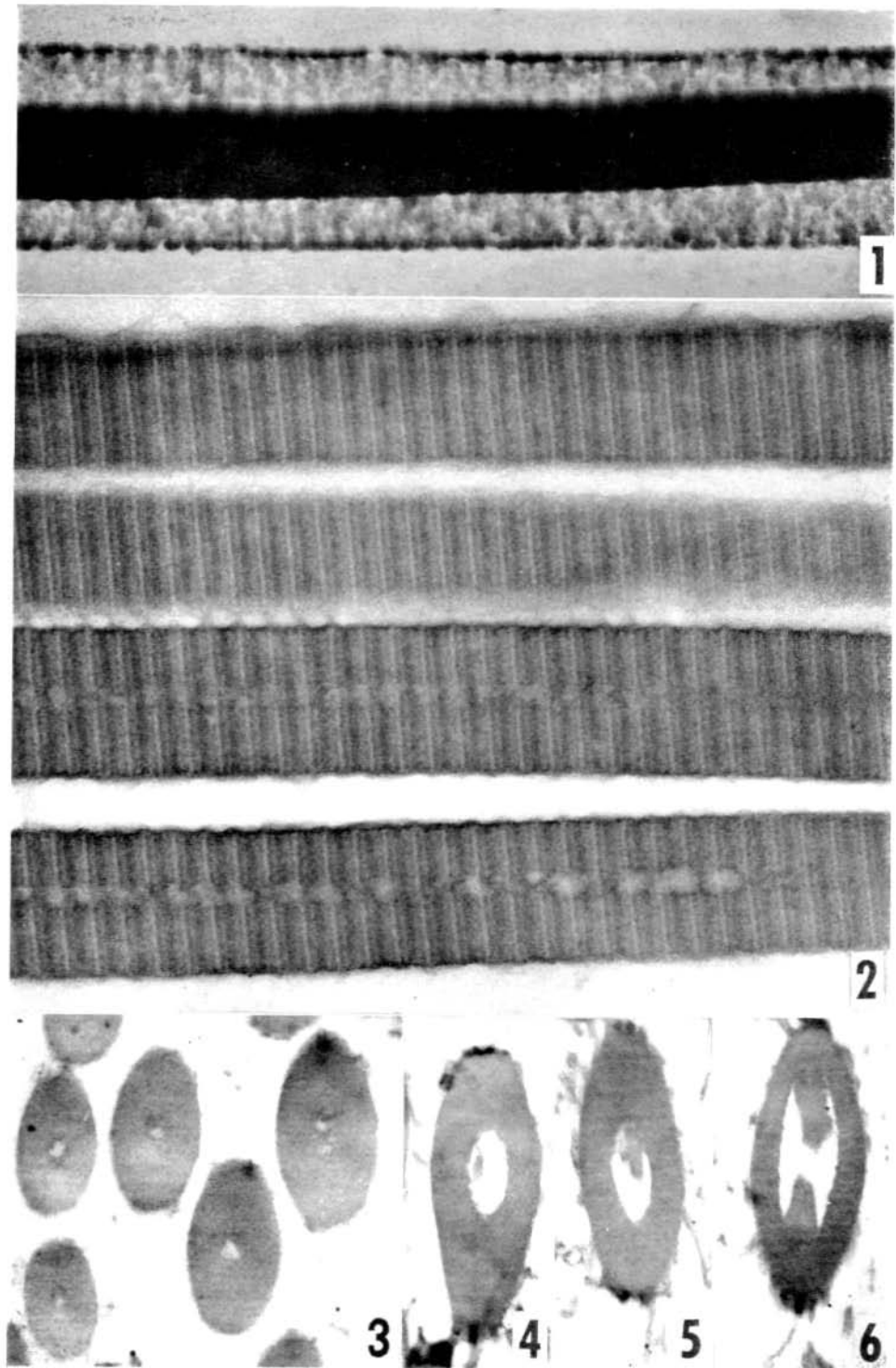


PLATE I

Fig. 1. *Cyathura* sp. Portion of sperm tail, showing indications of transverse banding in flattened periaxial sheath (dried mount). $\times 50,000$.

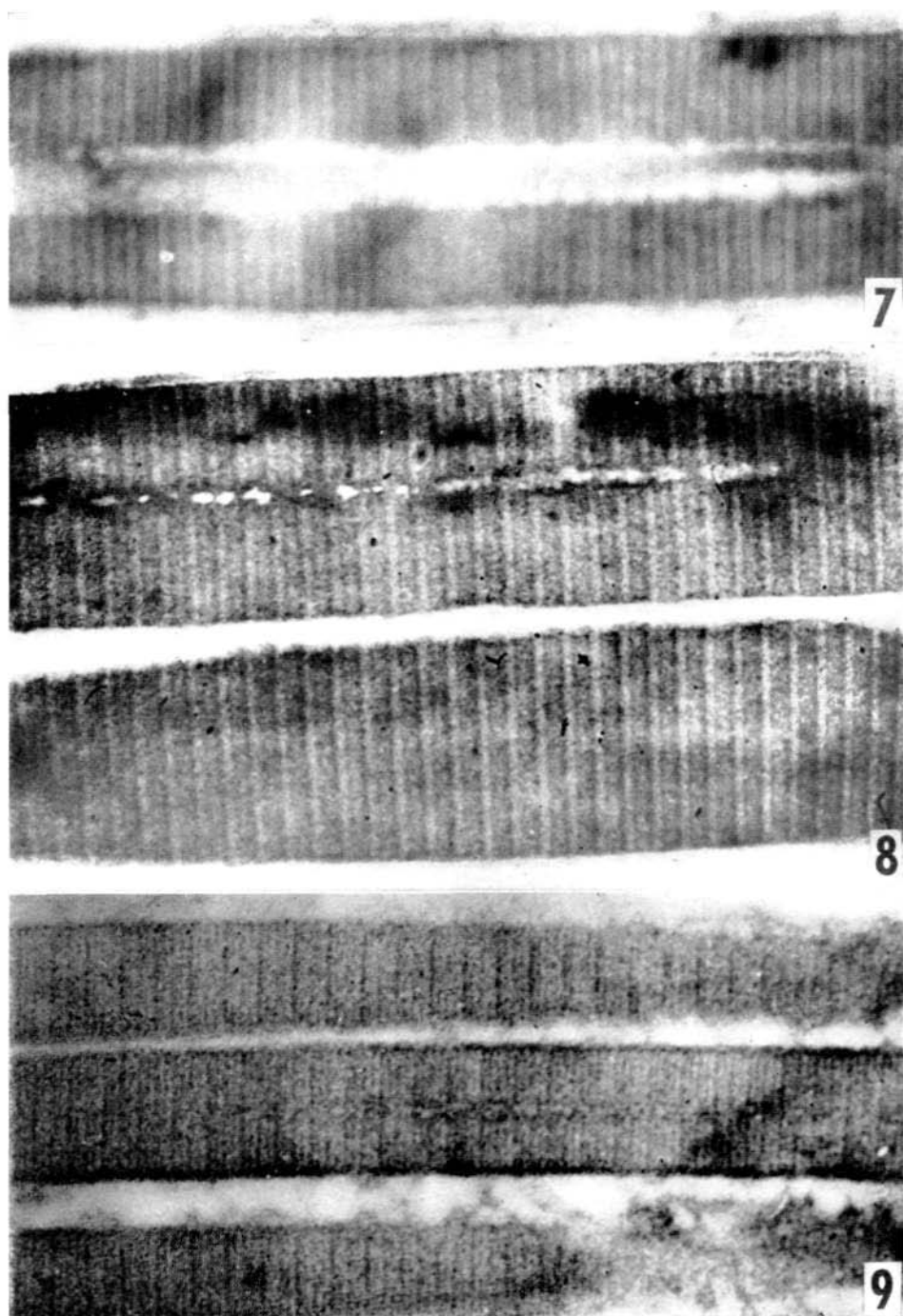


PLATE II

- Fig. 7. *Idotea balthica*. Portion of sperm tail, proximal region (longitudinal section). $\times 95,000$.
Fig. 8. *Idotea balthica*. Portions of sperm tails, distal region (longitudinal section). $\times 82,000$.
Fig. 9. *Oniscus asellus*. Portions of sperm tails (longitudinal section). $\times 76,000$.