ACCADEMIA NAZIONALE DEI LINCEI

Reprint from **Quaderno N. 137**

Comparative spermatology

Proceedings of the Ist International Symposium

(Rome–Siena, 1–5 July 1969)

Edited by BACCIO BACCETTI

C. BEDINI (*) and F. PAPI (*)

PECULIAR PATTERNS OF MICROTUBULAR ORGANIZATION IN SPERMATOZOA OF LOWER TURBELLARIA (**)

SUMMARY. — The acoel *Convoluta psammophila* presents spermatozoa with two axial units, which show 9 + 0 fibrils for the greatest part of their length and 9+2 fibrils for a short extent at the distal end. The spermatozoa of the macrostomids *Promacrostomum gieysztori* and *Macrostomum retortum* are motile, lack axial units or flagellar-like structures but show two series of microtubules longitudinally arranged.

RIASSUNTO. — L'acelo *Convoluta psammophila* presenta spermatozoi con due filamenti assiali che mostrano organizzazione microtubulare a modello 9+0 per la maggior parte della loro lunghezza e 9+2 per un breve tratto alla estremità distale. Gli spermatozoi dei macrostomidi *Promacrostomum gieysztori* e *Macrostomum retortum* sono mobili, mancano di filamenti assiali o strutture simili a flagelli ma mostrano due serie di microtubuli ad andamento longitudinale.

In sperm flagella of all platyhelminths studied by electron microscopy (10 species of turbellarians, 6 trematodes and 4 cestodes) (AFZELIUS, 1964; BONSDORFF and TELKKÄ, 1965; CHRISTENSEN, 1961; GRESSON, 1962; GRESSON and PERRY, 1961; HENDELBERG, 1967; HERSHENOV, TULLOCH and JOHNSON, 1966; KLIMA, 1961; SHAPIRO, HERSHENOV and TULLOCH, 1961; SILVEIRA and PORTER, 1964; TULLOCH and HERSHENOV, 1967) an identical 9+1 arrangement, i.e. a single central tubule surrounded by nine peripheral doublet tubules, had been noted.

It has been observed that it is unlikely for such a character to have arisen independently in different groups of platyhelminths, and therefore it could be considered as an original character for the phylum (HENDELBERG, 1967). The report by COSTELLO *et al.* (1969) recently published by *Science* according to which an acelous turbellarian, *Childia groenlandica*, has spermatozoa with a 9+0 arrangement, is all the more interesting as acoels, which had not been studied under this point of view, are regarded by various AA. (see for references DOUGHERTY, 1963; IVANOV, 1968) as primitive forms in the evolution of all other platyhelminths.

Two arrangements of sperm microtubules, new for the platyhelminths, were observed, however, in another acoel, *Convoluta psammophila* and in two species of the close order of Macrostomida, *Macrostomum retortum* and *Promacrostomum gieysztori*.

(*) Institute of General Biology, University of Pisa, Italy.

(**) Supported by a grant from Consiglio Nazionale delle Ricerche.

Electron microscope observations were made on sections of entire animals fixed in 1% osmium tetroxide buffered with Millonig 0.2 M. or in 2.5% glutaraldeyde in cacodilate buffer and postfixed in osmium tetroxide. Embedding in Epon 812—Araldite mixture, and sections stained with uranyl acetate and lead citrate.

Convoluta has motile spermatozoa about 270 μ long, ribbon shaped and tapering somewhat abruptly to the anterior end and gradually to the posterior one.

In the sperm region, behind the nucleus, two axial units are present in the cytoplasm; they run close to the plasma membrane as far as the posterior end. Here, the tail branches into two short tips, into both of which the axial unit continues. In this two–branched region, the axial units consist of nine peripheral doublets and two central tubules, while elsewhere only nine peripheral doublets occur (Fig. 1 and 2).

Both axial units, each about 2300 Å in diameter, are separated from the other cytoplasmic organelles and inclusions by a membranous structure (Fig. 1: *M*), which runs medially along the entire length of the axial unit and side by side with it. The microtubular doublets have a diameter of about 380 Å.

In the median region of the sperm, centrally, 10 microtubules are also present. This latter have frequently organized themselves into a third atypical axial complex.

In spermatids (Figs. 3 and 4), axial units are incorporated for all their length into a cavity completely limited by a membrane (Fig. 3: *M'*). This is again surrounded by a membranous system (Fig. 3: *M''*), which appears omogeneous and strongly electron–dense on the side facing the external wall of the cell, while being elsewhere risolvable into more laminar units. 12–14 microtubules are contained between the membrane *M'* and the membranous system *M''*, in the internally facing hemicycle. The relative extension of 9+0 and 9+2 tracts is probably dependent on the spermatids stage. The 9+0 one is more distal but sometimes may be found even at the nucleus level (Fig. 3). Other longitudinally lying microtubules, in addition to those already described, are present under the plasma membrane.

Promacrostomum giesztori has thread-shaped motile spermatozoa, about 45 μ long, bearing two stiff bristles at boundary between 1st and 2nd third (PAPI, 1951). *Macrostomum retortum* has identically shaped, although about 70 μ long, spermatozoa. The E. M. observations have not shown structural differences between the two species.

The spermatozoa are lacking axial units or other structures identifiable with orthodox flagella. A microtubular sheath starting from the nucleus runs up to the tail tip under the plasma membrane. This discontinues for a short way on two opposite sides (Fig. 5). The microtubules, about 250 Å in diameter, run parallel to each other and to the longitudinal axis of the sperm. In the region close to the nucleus 60–70 microtubules rather spaced from each other are counted, and at the caudal end 25–30 closely lined ones. In the middle regions transitional figures between both conditions are present.

The two bristles are thin cytoplasmic expansions with a homogeneous strongly electron-dense core (Fig. 6). In their longitudinal sections these bristles show, under the plasma membrane, a regular succession of cross-cut microtubules. This pattern can be interpreted as a close microtubular coiling around the central core.

As far as we know, the mixed 9+0 and 9+2 pattern of *Convoluta* has not been described either in turbellarians or in other animals. This pattern appears to arise from a reduction in the extension of the central tubules and this process might have led to the 9+0 pattern of *Childia*. The sperm structure of *Promacrostomum* and of *Macrostomum* recalls that of some arthropods (ROBISON, 1966). In this instance it might be derived from that of acoels as a consequence of loss of axial units and preservation, also in the mature sperm, of the other extraaxial microtubules. The present findings suggest that it is probably a hasty decision to use the structural characters of spermatozoa for phylogenetic speculations on platyhelminths at least until more species belonging to the main orders can be studied.

REFERENCES

AFZELIUS B. (1964) – *Cellen W and W-serien 57*. Wahlström and Widstrand, Stockholm.

BONSDORFF C. H. and TELKKÄ A. (1965) – *The spermatozoon flagella in* Diphyllobothrium latum (*Fish tapeworm*). « Z. Zellforsch. », 66, 643–648.

CHRISTENSEN K. (1961) – *Fine structure of an unusual spermatozoon in the flatworm* Plagiostomum. « Biol. Bull. », 121, 416.

COSTELLO P. P., HENLEY C. and AULT C. R. (1969) – *Microtubules in spermatozoa of* Childia (*Turbellaria, Acoela*) *revealed by negative staining*. « Science », 163, 678–679.

DOUGHERTY E. C. (Ed.) (1963) – *The lower metazoa*. Univ. Cal. Press, Berkeley.

GRESSON R. A. R. (1962) – *Spermatogenesis of a cestode*. « Nature », 194, 397–398.

GRESSON R. A. R. and PERRY M. M. (1961) – *Electron microscope studies of spermateleosis in* Fasciola hepatica L. « Exp. Cell Res. », 22, 1–8.

HENDELBERG J. (1967) – *On different types of spermatozoa in Polycladida, Turbellaria*. « Ark. Zoologi », 18, 267–304.

HERSHENOV B. R., TULLOCH G. S. and JOHNSON A. D. (1966) – *The fine structure of trematode* (Haematoloechus medioplexus) *sperm tails*. « Trans. Amer. Microsc. Soc. », 85, 480–483.

IVANOV A. V. (1968) – *Proiskhoždenie mnogokletočnykh životnykh*. Nauka, Leningrad.

KLIMA J. (1961) – *Elektronen mikroskopische Studien über die Feinstruktur der Tricladen* (*Turbellaria*). « Protoplasma », 54, 101–162.

PAPI F. (1951) – *Ricerche sui Turbellari Macrostomidae*. « Arch. zool. ital. », 36, 289–340.

ROBISON W. G. (1966) – *Microtubules in relation to the motility of a sperm syncytium in an armored scale insect*. « J. Cell Biol. », 29, 251–266.

SHAPIRO J. E., HERSHENOV B. R. and TULLOCH G. S. (1961) – *The fine structure of* Haematoloechus *spermatozoan tail*. « J. Biophis. Biochem. Cytol. », 9, 211–217.

SILVEIRA M. and PORTER K. R. (1964) – *The spermatozoids of flatworms and the microtubular system*. « Protoplasma », 59, 240–265.

TULLOCH G. S. and HERSHENOV B. R. (1967) – *Fine structure of platyhelminth sperm tail* (Haematoloechus medioplexus). « Nature », 213, 299–300.

Explanation of figures

Fig. 1. – Spermatozoa of *Convoluta* in cross sections, showing the two lateral axial units (1 and 1') lacking central fibrils, the membranous structure (*M*) and the third atypical axial complex (*C*). ×24.000.

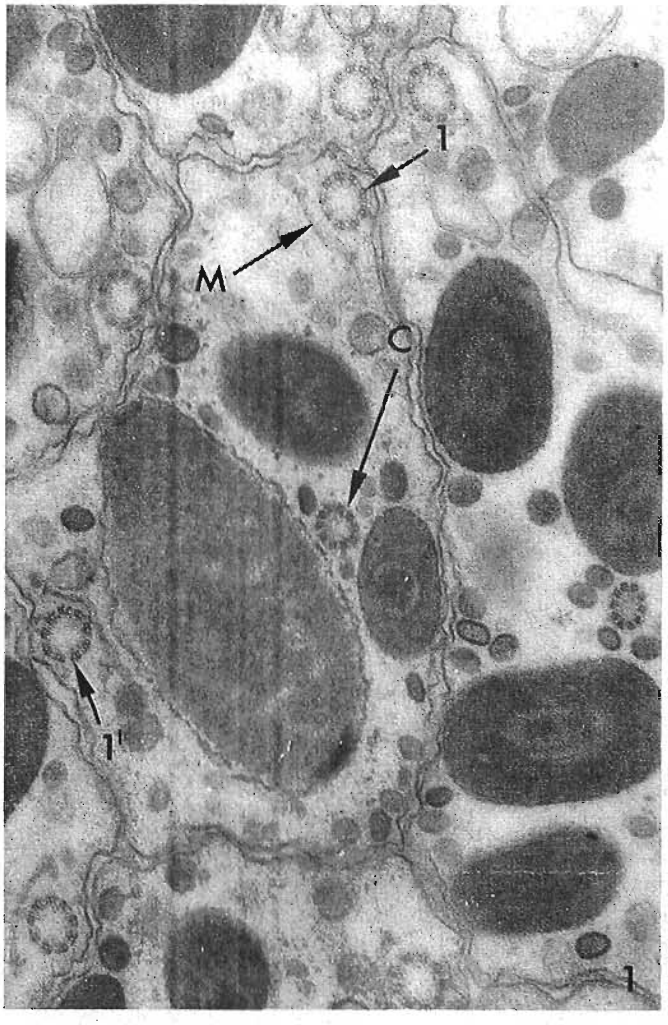
Fig. 2. – Distal part of spermatid tail of *Convoluta* showing the 9+2 pattern of the axial complex. ×65.000.

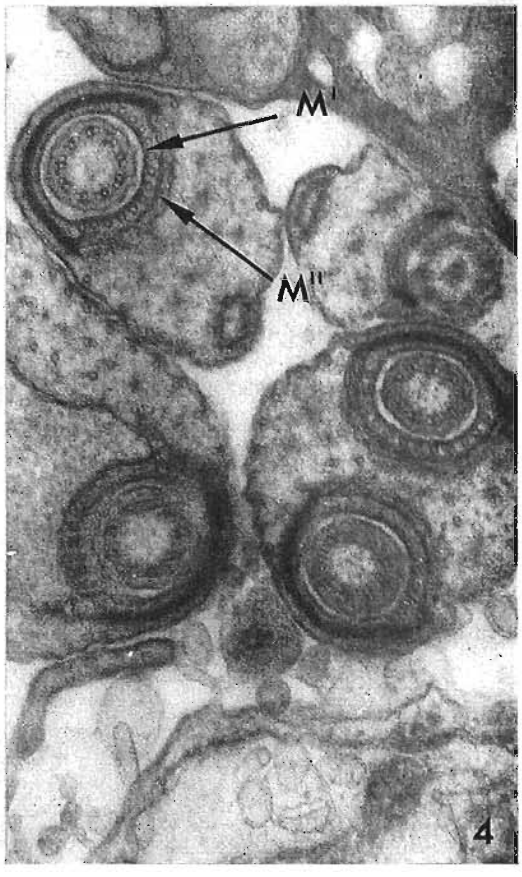
Figs. 3 and 4. – Cross sections of *Convoluta* spermatids. Note the 9+0 and 9+2 arrangement of the axial units. *M'*, *M''*: membranous structure and system. Fig. 3 ×52.000; Fig. 4 ×42.900.

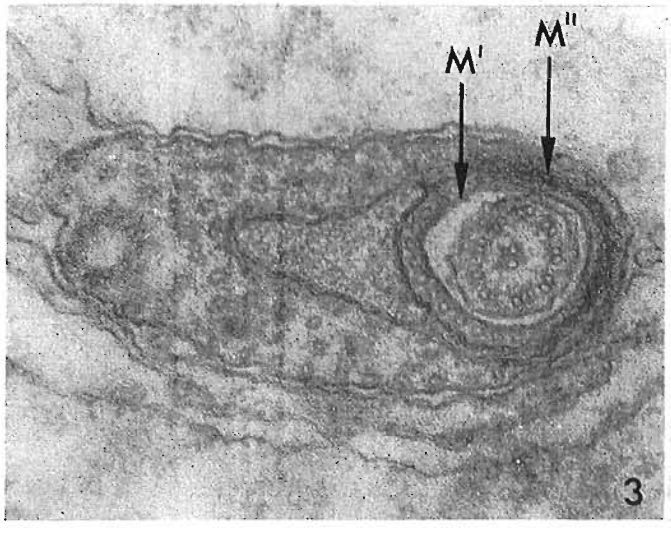
Fig. 5. – Proximal and distal region of sperm tails of *Macrostomum* cross – sectioned showing microtubules. Oblique sections of bristles (*B*) are visible. ×16.000.

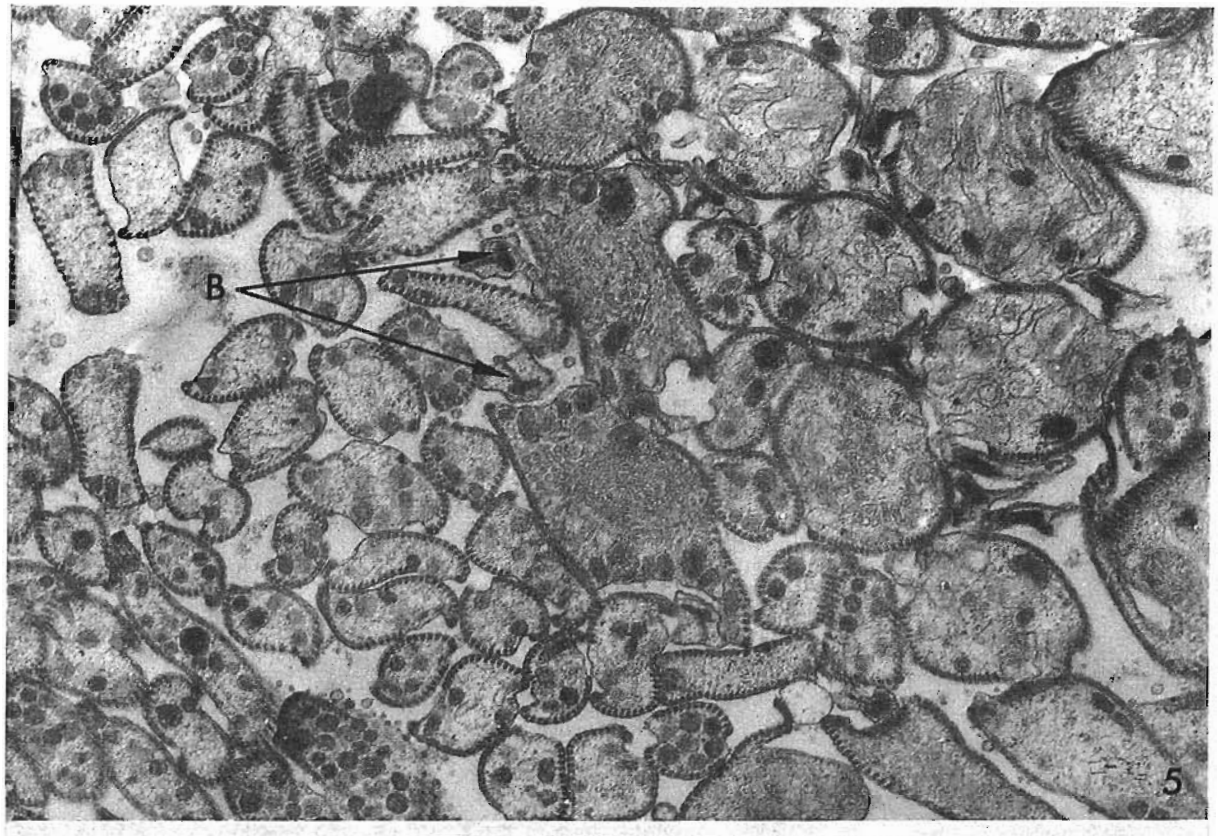
Fig. 6. – High magnification of a portion of bristles (*B*) showing the dense internal core and the microtubular coiling under the plasma membrane. ×48.000.

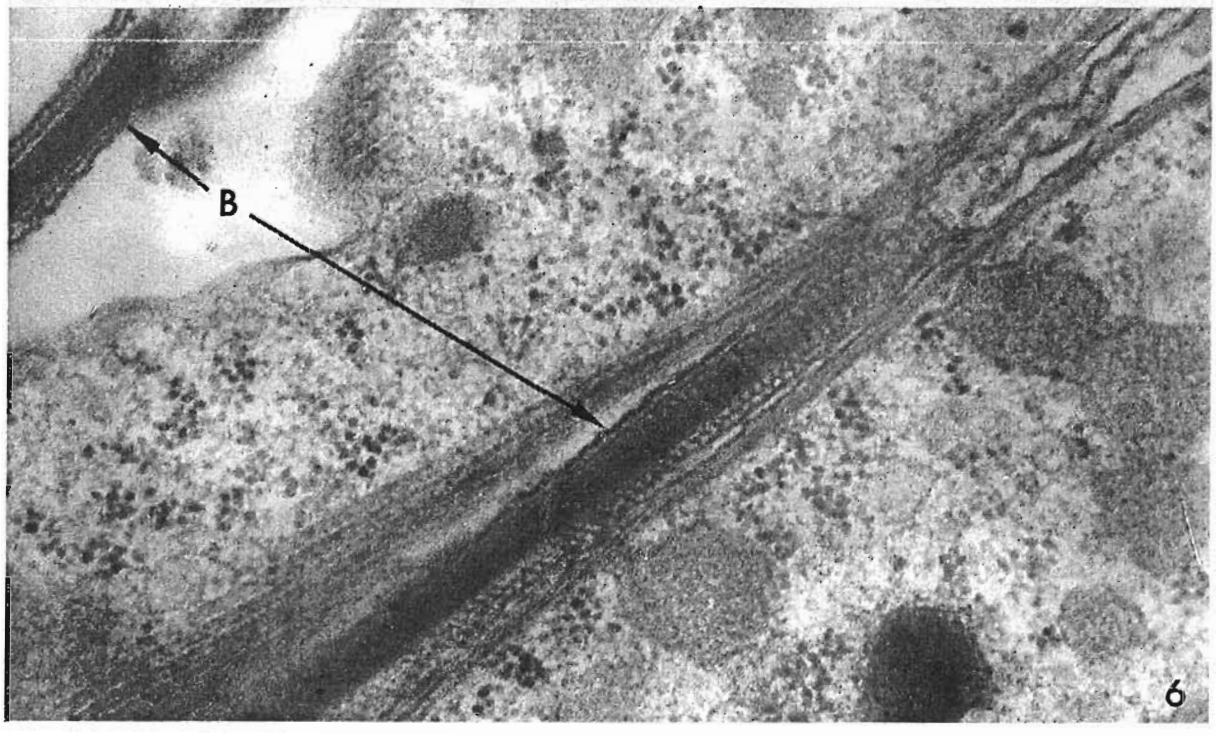
C. BEDINI and F. PAPI - *Peculiar patterns of microtubular organization in spermatozoa of lower turbellaria.*








B
5


B
6