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The spermatozoon of Pseudoscorpions (Arachnida)

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RIASSUNTO

Questo studio riporta una indagine sulla struttura dello spermatozoo degli Pseudoscorpioni, basata essenzialmente su una tecnica di fissazione che consente una migliore conservazione dei microtubuli. In questo modo sono stati identificati tre modelli assonemali, il più semplice dei quali è stato attribuito alle specie delle famiglie *Chthoniidae* e *Neobisiidae*. I dati ottenuti sono stati utilizzati per una discussione sui rapporti filogenetici all'interno degli Pseudoscorpioni e nella classe degli Aracnidi.

Parole chiave: Spermatozoi, Psudoscorpioni, Filogenesi.

ABSTRACT

The present study describes new evidences on the ultrastructure of pseudoscorpion spermatozoa after tannic acid fixation. Three main axonemal models were observed: the simplest was found in *Chthoniidae* and *Neobisiidae*. *Cheiridiidae, Atemnidae, Cheliferidae* and *Chernetidae* have a 9+2 axoneme, surrounded by accessory sheaths, while *Garypiidae* and *Olpiidae* have a 9+9+2 axoneme. The phylogenetic significance of these results was discussed.

Key words: Spermatozoa, Pseudoscorpions, Phylogeny.

Introduction

Reproduction in Pseudoscorpions is performed by indirect transfer of spermatozoa through spermatophores (WEYGOLDT, 1969). Spermatophores, show different degree of complexity, and contain a variable number of encysted spermatozoa. All the pseudoscorpion spermatozoa examined so far belong to the coiled-flagellate type (SOKOLOV, 1926; NESTER, 1932; TUZET *et al.*, 1966; LEGG, 1973; BOISSIN, 1974; CALLAINI & DALLAI, 1984, 1987), a model which is also found in Araneids,

Uropygi, Amblypygi and Ricinulei among Arachnida, and have essentially the same basic structure: an elaborated acrosomal complex that partially surround a condensed cylindrical nucleus, a pair of centrioles, elongated mitochondria, and a long flagellum.

The main goal of this work is to give new insights on sperm ultrastructure after preparation of pseudoscorpion spermatozoa with a new fixative (DALLAI & AFZELIUS, 1990) that is known to give better preservation of the microtubular structures.

Materials and methods

Pseudoscorpion species examined were: *Chthonius ischnocheles*, *Chthonius tetrachelatus*, *Neobisium muscorum*, *Roncus lubricus*, *Acanthocreagris italica*, *Garypus levantinus*, *Geogarypus nigrimanus*, *Garypinus dimidiatus*, *Amblyolpium dollfusi*, *Calocheiridius olivieri*, *Apocheiridium ferum*, *Rhacochelifer maculatus*, *Hysterochelifer tuberculatus*, *Pselaphochernes lacertosus*, *Allochernes wideri*, *Atemnus politus*. Males were dissected in phosphate buffer and testes were fixed according to DALLAI & AFZELIUS (1990), to improve the visualization of microtubules. For freeze-fracturing the material was fixed for 30 min in 5% glutaraldehyde and 4% paraformaldehyde in cacodylate buffer and infiltrated with a graded series of glycerol solutions. Fixed material was cooled with a graded series of glycerol solutions. Fixed material was cooled with liquid nitrogen and fractured at -115° C in a Balzer BAF 301 freeze-etching device. Samples were observed and photographed with a Philips CM10 electron microscope.

Results

Acrosomal complex

The formation of the acrosome is a very complex process in pseudoscorpions, that in every species studied so far depends on the secretory activity of a large Golgi complex. The importance of Golgi activity during spermatogenesis first emphasized by SOKOLOW (1926) and NESTER (1923) at the light microscope, has been confirmed by transmission electron microscopical observations (BOISSIN & MANIER, 1967; WERNER & BAWA, 1988; DALLAI & CALLAINI, 1990).

The process of acrosome formation starts with the positioning of a large Golgi complex, constituted by several densely packed cisternae, over the spermatocyte nucleus (Fig. 1). The secretory activity of these cisternae leads to the production of a large number of small vesicles that accumulate between the nucleus and the Golgi apparatus. Later, a large irregular vesicle filled with filamentous material takes contact with the anterior region of the early spermatid nucleus. The acrosomal material progressively increases its density in the axial part, and pushes off the acrosomal membrane in such a way that a kind of hook is formed. Later on the acrosomal membrane in close contact with the nuclear membrane forms an invagination in its central region and, at the same time, the nuclear envelope also begins to form an endonuclear cavity. This subacrosomal cavity, in which progressively amorphous dense material accumulates, corresponds to the perforatorium.

During the formation of the primitive acrosomal vesicle the nucleus elongates and chromatin condenses in the apical region. Pore complexes, absent from nuclear envelope surrouding the condensed chromatin, are instead very numerous in the region overlapping the chromatin-free nucleoplasm (Fig. 2); it is suggesting that this region is still involved in metabolic traffic. The secretory activity of the Golgi apparatus also leads to the formation of a peculiar kind of ribbon that starts from the acrosomal vesicle and surrounds the nucleus in helicoidal fashion, moving toward its basal end.

The proces of acrosome formation is essentially the same in all species of pseudoscorpions studied so far. However, the *Geogarypus* spermiogenesis develops with interesting variations, dealing with the great extension of the acrosomal helix. This structure forms at least three coils in the encysted cell (Fig. 3). In cross section the acrosomal material is stratified in layers to give origin to several laminae according to different planes; this feature is reminiscent of a crown (DALLAI & CALLAINI, 1983, 1990) (Fig. 4).

Axoneme

In early spermatocytes there is a single centrosome constituted by a pair of orthogonally arranged centrioles (Fig. 7). The centrioles move apart and organize the microtubules of the mitotic spindle that leads to the formation of two sister spermatids. In cross section, instead of the conventional triplets of most metazoan centrioles, only doublet micro-

tubules with A and B subtubules were found (Fig. 8). C-tubules, when visible are incomplete, and are formed by 3-4 protofilaments disposed in hook-like fashion. Tubules are embedded in the dense pericentriolar material. These aberrant centrioles persists in the young spermatid, when the pericentriolar material begins to organize dense projections, radially oriented (Fig. 9). At sperm maturity two coaxial, or obliquely arranged centrioles of different size are present (Fig. 10). The short proximal centriole is located in a nuclear invagination. The distal centriole, from which the axoneme originates, shows the usual feature of nine complete triplets of A- B-, and C-tubules with 13, 10, and 10 protofilaments respectively (Fig. 11). Each triplet is embedde in a dense mass and is connected with an axial rod by a radial link. This structure gradually transforms in the basal part of the axoneme, and is characterized by 9 doublets devoid of arms and by two central tubules (Fig. 12). The basic structure of the axoneme, which is enclosed in a more or less evident flagellar tunnel, is essentially the same in the pseudoscorpion species examined so far. This consists of the well known 9+2 tubular pattern with well evident outer and inner dynein arms, radial links, nexin bridges, and dense central sheath that envelopes the inner pair of microtubules (Fig. 5). A- and B-tubules are formed by the conventional 13 and 10 pfs, respectively. The position of dynein arms on A subtubule is that described in many other flagellar axonemes, with outer arms projecting from the gap between pf. n° 13 and 14 and inner arm in that one between pf. n° 16 and 17 (Fig. 6).

Little differences in axonemal morphology were recognized in the spermatozoa of the species that we have examined and three models can be described. The most simple model is that represented by tha axoneme of *Chthonius*, which is also found in *Neobisium*, *Roncus*, and *Acanthocreagris*. In these cases the axoneme and the flagellar tunnel are only surrounded by cell membranes. Whithin this model *Garypinus* and *Amblyolpium*, show a greater complexity of the axonemal structure due to the presence of a fibrous cytoplasmatic dense material closely apposed to the plasma membrane (Fig. 13).

The second model is found in several *Cheliferidae* species where a dense fibrous material forms a compact envelope that surrounds the flagellar tunnel. A thin sheath is also present around the axoneme (Fig. 17). The flagellar sheath is well developed in *Chernetidae* and *Antemidae* species, whereas it is lacking in *Apocheridium* (Fig. 16), and incomplete in *Rhacochelifer* and *Hysterochelifer*. In these latter species

the dense material adhering to the inner face of the flagellar mambrane, at the distal portion of the flagellum is fragmentated and reduces to nine small patches located externally to the peripheral axonemal doublets (Figs. 18, 19).

The third axonemal model is found in *Geogarypus*, *Garypus*, and *Calocheiridius* (Fig. 14). In the proximal half of the axoneme of these species nine thin bundles of electrodense fibres adhere externally to the peripheral doublets, forming accessory structures (Fig. 15).

Discussion

Spermiogenesis in pseudoscorpions ends with a peculiar coiling process, that occurs before the spermatozoa are discharged in the lumen of the testis, where they appear surrounded by a secretory sheath. Therefore, spermatozoa are immotile in deferentes, but according to BOISSIN (1970), they become motile in female genital tract when tail is unrolled and free to beat in the external medium. The shared coiled condition leads to an apparently morphological uniformity of pseudoscorpion spermatozoa, that however, differ each other for the dimensions and the relative position of the several components. On the basis of the relative position of nucleus and mitochondria LEGG (1973) recognizes three morphological groups, where the discoidal model found in the families *Neobisiidae* and *Cheiridiidae*, may represents the ancestral condition from which the more complex types, found in *Chthoniidae*, *Cheliferidae* and *Chernetidae*, were derived. Our study, mainly based on ultrastructural features of the flagellum, confirms the primitive condition of *Neobisiidae* sperms, and the more complex condition found in *Chernetidae* and *Cheliferidae*, but also shows the simple condition of the axoneme found in *Chthoniidae*. It is interesting to note that this increase in sperm complexity also follows a similar increase in the complexity of the spermatophore transfer and sexual behavior (WEIGOLDT, 1966).

Special remarks deserves the systematic position of *Cheiriididae*. Recently, HARVEY (1992) by a clastidic analysis revised the phylogeny of pseudoscorpions and proposed a cladogram were *Cheiridiidae* belong to the superfamily *Garypoidea*. This is in disagreement with previous opinions of CHAMBERLIN (1931) and BEIER (1932a, b) who retained *Cheiridiidae* more closely related to *Chernetidae*, *Atemnidae* and *Cheliferidae* rather than to *Garypidae*.

Our ultrastructural data indicate that a dense fibrous sheath around the flagellar tunnel is present in *Withidae*, *Cheliferidae*, *Chernetidae*, *Atemnidae*, and *Cheiridiidae*. Such occurence may suggest that this character is a synapomorphy for these families, which, as consequence, are closely related. A fibrous sheath was also found in *Cheiridiidae*. On the other hand *Garypidae* and *Geogarypidae* have a 9+2 axoneme with accessory fibres. This model also found in *Olpiidae* is not present in *Cheiridiidae*. Thus the position of *Cheiridiidae* among *Garypoidea* is not supported by spermatological data.

It is now well accepted that sperm morphology represents an useful tool for phylogenetic analysis. WEIGOLDT & PAULUS (1979) carefully considered sperm morphology in their attempt to clarify systematic relationships within Chelicerata. However, there are many indications suggesting that the systematic value of comparative spermatological data has to be limited to low-ranging taxa (WIRTH, 1984).

It is likely that the filiform spermatozoa of Scorpions with the 9+1 or 9+0 axonemal pattern represents the type closest to the sperm of Xiphosura. Araneids, Uropygi, and Amblypigi have similar coiled-flagellate cells, with a peculiar 9+3 axoneme. This supports the common opinion that *Araneae* and Pedipalpi are closely related groups (ALBERTI, 1990).

The axoneme of Araneids mainly differs from that of Pseudoscorpions, for the presence of three central tubules, and a differently organized central sheath, consisting of spokes projecting from the three central tubules. The flagellum originates from a distal centriole arranged in a posterior nuclear invagination and constituted by nine doublets (the C-tubule being lacking) and three central tubules (DALLAI, WITALINSKY & AFZELIUS, in prep.). The coaxial orientation of distal and proximal centrioles seems a plesiomorphic character in all Chelicerata.

Opiliones and Acarina have aflagellate spermatozoa. This character appears as a secondary loss occurred independently in the two orders. Opiliones have disk-shaped or fusiform aflagellate sperms, but an axoneme is present in the spermatid (JUBERTHIE *et al.*, 1976). Acarina display cylindrical sperm cells that can actively move, perhaps by an actin-based mechanism. According to FELDMAN-MUSHAM & FILSHIE (1976), the cortical region of tick spermatozoa is characterized by longitudinal bundles of dense fibers, surrounded by a fine-fibrillar coat located below a series of external protrusions. Several ultrastructural studies suggested that the motor for sperm movement is located in the

superficial cytoplasm rather than in surface protrusions, but until now the presence of actin in tick sperms was not demonstrated.

WITALINSKY & DALLAI (in prep.), studying the spermatozoon of the soft tick *Argas polonicus*, provide evidence of the presence and distribution of actin in thick spermatozoa as well as its participation in the progressive gliding motion.

Ricinulei, the retained sister group of Acarina, display the primitive condition represented by a coiled-flagellate cell with a 9+2 long axoneme (ALBERTI & PALACIOS-VARGAS, 1984).

The loss of the flagellum is occurred several times in Chelicerata, and Solifugida also have aflagellate spermatozoa as Acarina Actinotrichida (ALBERTI, 1980). Solifugida, despite the lack of axoneme, are retained the sister-group of Pseudoscorpions on the basis of several synapomorphies (WEIGOLDT & PAULUS, 1979; SHULTZ, 1990).

Coming back to pseudoscorpions, let us now compare the length of the acrosome, nucleus and flagellum in *Geogarypus*, and other species examined so far, it is evident that in *Geogarypus*, the acrosomal structure achieves a great development (DALLAI & CALLAINI, 1983; CALLAINI & DALLAI, 1984, 1987). For istance, in a common species as *Chthonius ischnocheles* the flagellum/acrosome ratio is about 18-20, while in *Geogarypus* it is 0.2-0.3. The extraordinary length of the acrosome, not detectable in the encysted sperm is well appreciated after unrolling (DALLAI & CALLAINI, 1990).

Because *Geogarypus* is the only pseudoscorpion with a giant acrosome, this character has to be considered as an autoapomorphy. We could speculate, however that the presence of a very short axoneme, might be considered as a sign of an evolutive tendency towards realization of an aflagellate spermatozoon, thus anticipating the aflagellate condition found in Solifugida.

Acknowledgement

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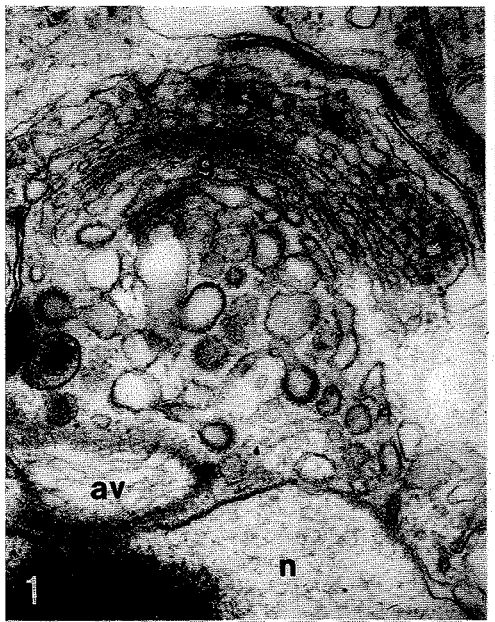
Fig. 1 - Acrosomal vesicle (av) formation in *Chthonius ischnocheles*. g, Golgi complex; n, nucleus. x 48.000.

Fig. 2 - Freeze-fracture replica of a *Chthonius ischnocheles* spermatocyte. Nuclear envelope shows pore complexes (pc) restricted to the posterior region. x 20.000.

Fig. 3 - Cross section of *Geogarypus nigrimanus* spermatozoa. ax, axoneme; m, mitochondria; a, acrosome. x 8.000.

Fig. 4 - Detail of the elaborated structure of the acrosome in *Geogarypus nigrimanus*. x 30.000.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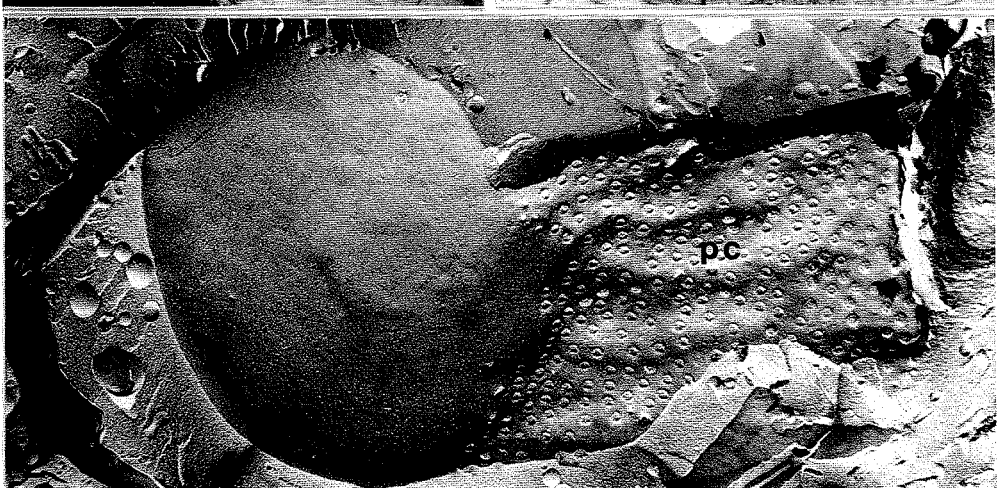
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Fig. 5 - Cross section through the axoneme of *Chthonius ischnocheles* late spermatids. x 120.000.

Fig. 6 - Detail of an axoneme of *Chthonius ischnocheles*. A- and B- subtubules are formed by 13 and 10 protofilaments, respectively; dinein arms (arrowheads) and spokes (arrows) are evident. x 230.000.

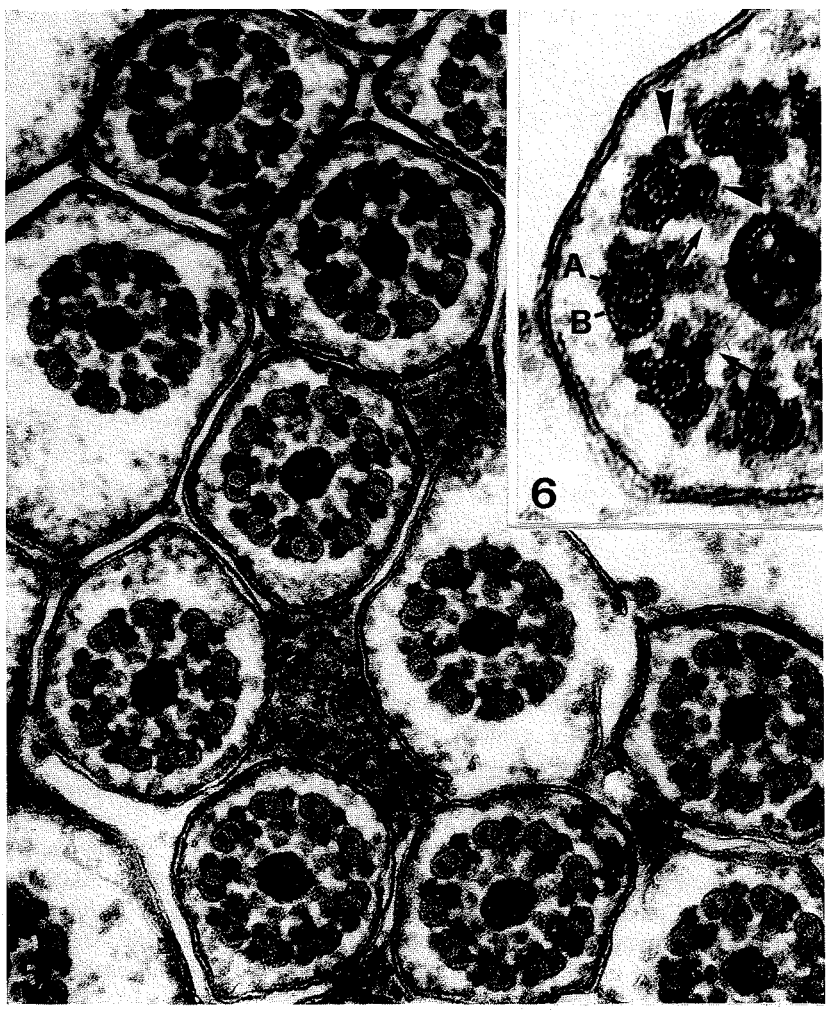
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Fig. 7 - Orthogonal centrioles in early spermatocyte of *Chthonius ischnocheles*. x 70.000.

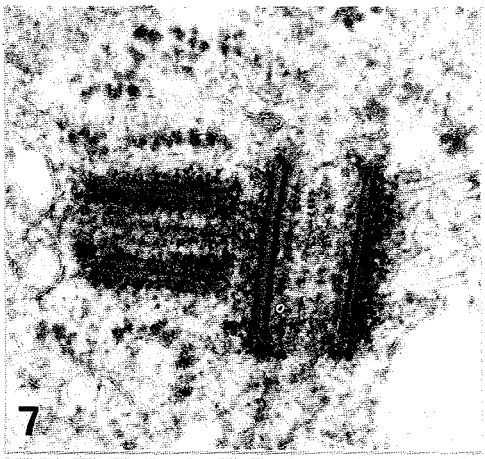
Fig. 8 - Detail of a centriole in a spermatocyte of *Chthonius ischnocheles*. A- and B-tubules are usually evident; rarely a hook of C-tubule can be seen (arrowhead). x 180.000.

Fig. 9 - Detail of a centriole in early spermatid of *Chthonis ischnocheles*. C-tubules are incomplete (arrowheads). x 180.000.

Fig. 10 - Longitudinal section through the nuclear region of a late spermatid of *Chthonius ischnocheles*. Note the almost coaxially oriented centrioles. x 30.000.

Fig. 11 - Cross section of the distal centriole in *Chthonius ischnocheles* spermatozoa, showing the usual feature of nine complete triplets. x 180.000.

Fig. 12 - Detail of the beginning of the axoneme of *Chthonius ischnocheles* showing 9 doublets still devoid of arms. x 180.000.



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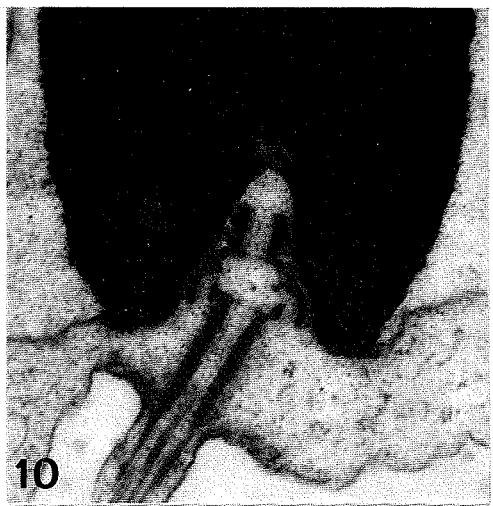

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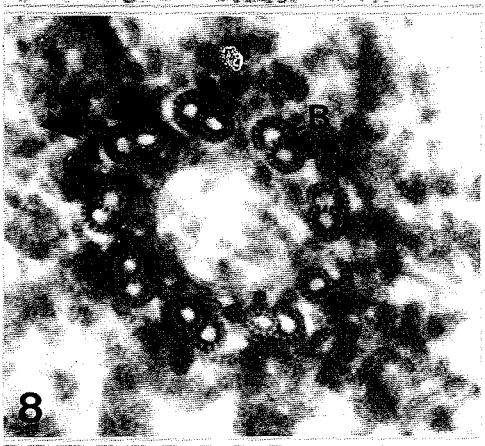

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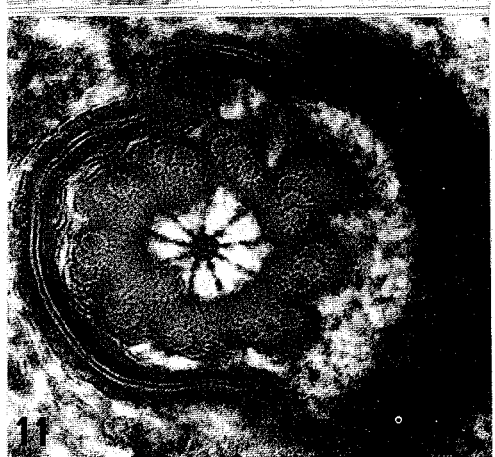

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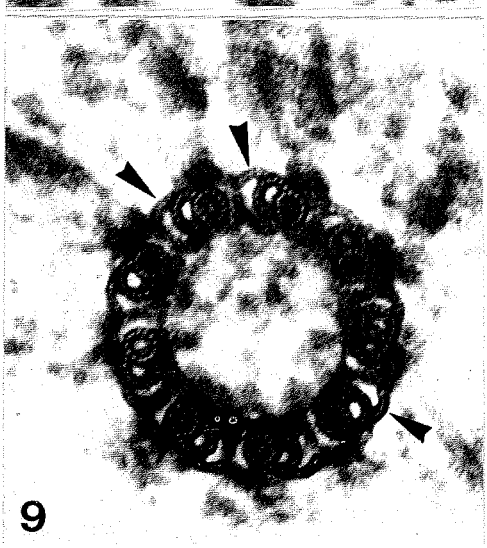

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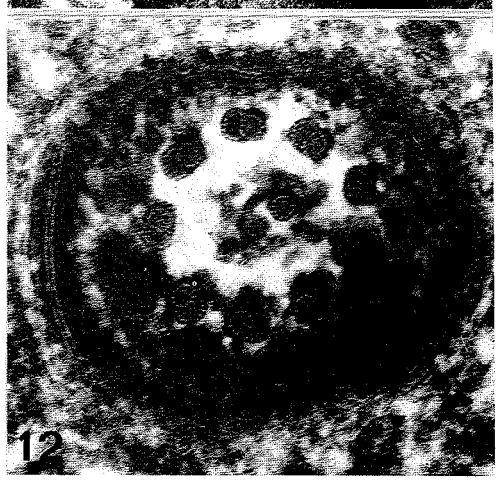

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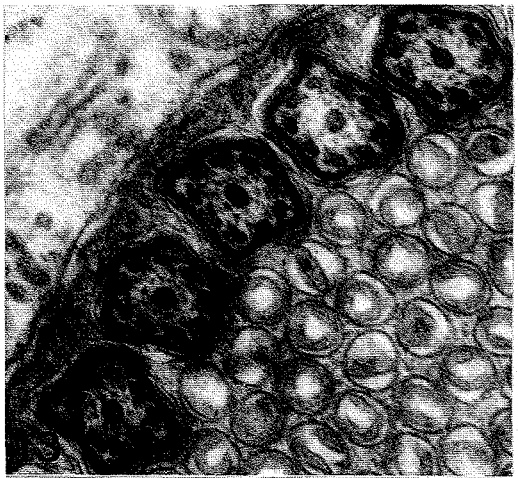
Fig. 13 - Cross-sectioned sperm axoneme from *Garypinus dimidiatus*. x 60.000.

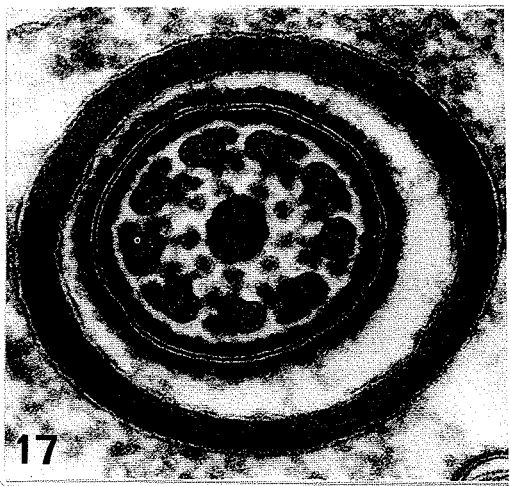
Fig. 14 - Axonemes from *Garypus levantinus*. x 40.000.

Fig. 15 - Detail of the axoneme of *Garypus levantinus* showing the accessory fibers (arrowheads). x 80.000.

Fig. 16 - Cross section of the mature sperm tail of *Apocheiridium ferum*. x 60.000.

Figs. 17 - 18 - 19 - Cross sections through different levels of *Rhacochelifer maculatus* axoneme. x 120.000.

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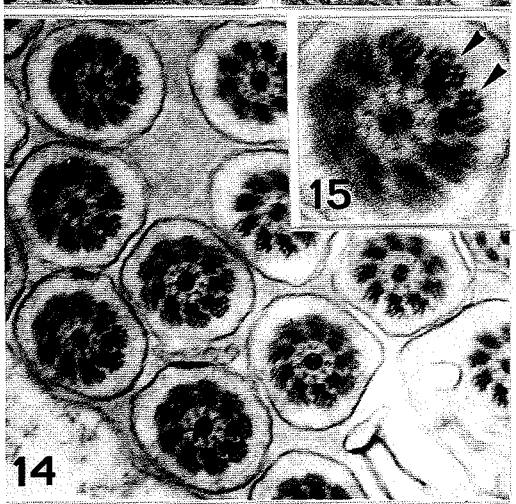
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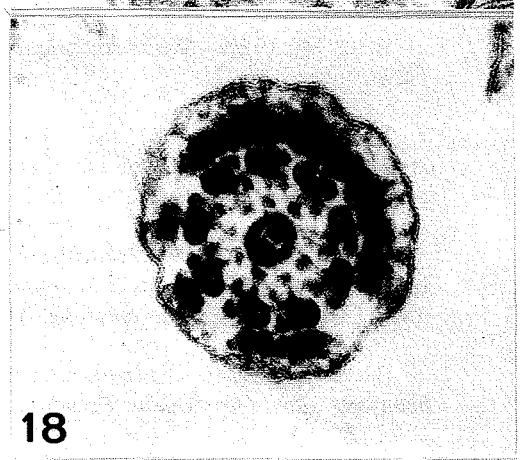
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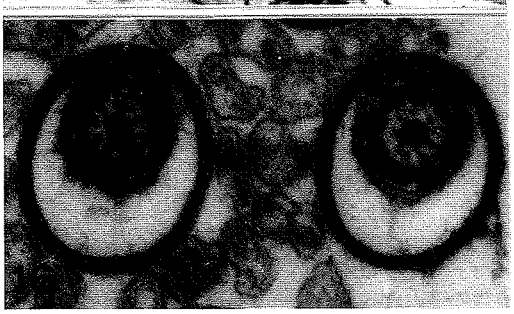

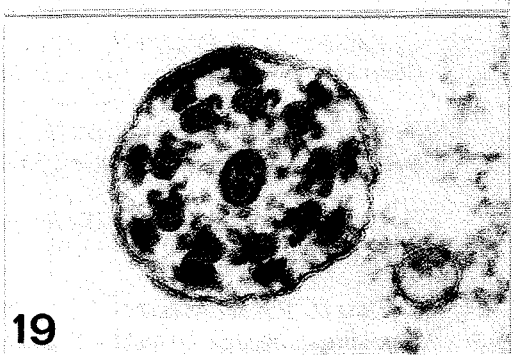
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