THE ULTRASTRUCTURE OF THE MULLET *MUGIL CUREMA* VALENCIENNES (TELEOSTEI, MUGILIDAE) SPERMATOZOA

Daura Regina Eiras-Stofella [1]
Waldemiro Gremski [2]
Sandra Maria Kuligowski [3]

ABSTRACT. The structure of the spermatozoon of **Mugil curema** Valenciennes, 1836 was studied using scanning and transmission electron microscopy. The spermatic head is rounded and formed by the nucleus containing granular chromatin, firmly packed resulting in a mass extremely electron dense. The acrossome is absent. The midpiece is characterized by the presence of two centrioles, a plasmatic canal, very few vesicles, and several mitochondria (9-10) with aproximately 0.50μm in diameter. The head and the midpiece are aproximately 1.56μm in diameter. The flagellum conforms to the 9 + 0 flagellar pattern near the transition region in its midpiece and is 9 + 2 from there on up to the distal region of the axoneme. The electron density in the A tubules 1, 2, 5 and 6 shows the asymetry of this spermatozoa. Its spermatic cell differs ultrastructuraly from those of other Mugilidae species mainly because it has the highest number of mitochondria.

KEY WORDS. Teleostei, Mugilidae,mullet, **Mugil curema**, ultrastructure, spermatozoa

In recent years, researchers have become increasingly interested in the ultrastrutural analysis of the Teleostei spermatozoa due to their wide variety of forms and structures.

Studies on the male gametes of five fish species belonging to the family Mugilidae revealed the existence of morphological intergeneric and interspecies differences (VAN HORST & CROSS, 1978; BRUSLE, 1981; EIRAS-STOFELLA & GREMSKI, 1991), which emphasized the point made by other authors who believe that this type of analysis is capable of distinguishing fishes of a same family even at specific level (MATTEI *et al.*, 1967; BACCETI *et al.*, 1984; MATTEI, 1991).

We now report results of ultrastructural analysis of the *Mugil curema* Valenciennes, 1836 spermatozoa in order to compare their morphology with that of other Mugilidae fishes.

1) Centro de Microscopia Eletrônica, Universidade Federal do Paraná, Caixa Postal 17003, 80051-970 Curitiba, Paraná, Brasil.
2) Departamento de Biologia Celular, Universidade Federal do Paraná, Caixa Postal 19031, 81531-970 Curitiba, Paraná, Brasil.
3) Bolsista de Iniciação Científica do CNPq.

MATERIAL AND METHODS

Twelve specimens of the *M. curema* (22.0-28.1cm) at an advanced stage of gonadal maturation were caught in January 1992 at the Pontal do Sul beach, Paraná, Brasil (approximately 25°35'S-48°22'W).

The sperm obtained from the gonads was fixed in a solution of 1.5% glutaraldeyde and post fixed in 1% osmium tetroxide, the buffer solution being 0.15M cacodylate.

For transmission electron microscopy (PHILIPS EM-300), the sperm was dehydrated in acetone, embedded in Polylite, sectioned with an ultramicrotome (SORVAL MR-1) and stained in 2% uranyl acetate and Reynold's solution. For scanning electron microscopy (PHILIPS SEM-505), the fixed semen was dehydrated in ethanol in order to reach the critical point (BALZERS CPD-010) and coated with a thin layer of gold (BALZERS SCD-030).

RESULTS

The *M. curema* spermatozoon consists of head, midpiece and a single flagellum. An undulant plasma membrane covers entirely the sperm cell.

Head. The head is rounded and has no acrossome (Figs 1a,b; 2a,d). The head including the midpiece has an average diameter of about 1.56μm.

The nucleus is situated at the apex of the head and its upper portion follows the same rounded shape of the head (Figs 2a,b). The basal portion presents a groove that forms the nuclear hilus (Figs 2b, 3). The nucleus, with a diameter of approximately 1.08μm, is covered by a double undulant nuclear membrane that involves a chromatin mass formed by extremely dense granules (Figs 2a,b; 3).

Midpiece. The midpiece comprises all of the structures situated between the nucleus and the plasmic canal and has its limits marked by the lamella which separates it from the flagellum (Fig. 2b).

Nine to ten mitochondria with a diameter of 0.59μm are situated between the plasmic canal and the nucleus (Figs 2a,b; 4). The mitochondria presents a double membrane and mitochondrial cristae in a plate arrangement (Figs 2a,b; 4).

The distal centriole was found near the transition region of the flagellum where a lamella separating this centriole from the flagellum was noticed (Fig. 2b). The characteristic structural configuration of the distal centriole is of the 9 + 0 type and is surrounded by a thin layer of dense material (Fig. 2b). The basal foot extends from the distal centriole towards the nucleus (Fig. 3). The proximal centriole is located near the nuclear hilus forming an oblique angle with the distal centriole (Fig. 2a). Few vesicles of various sizes were observed in the cytoplasm (Fig. 2s).

The plasmic canal is formed by the plasma membrane which invaginated towards the head defining the separation between the flagellum and the mitochondria. It is strangthened by a small quantity of dense material (Figs 2a,b).

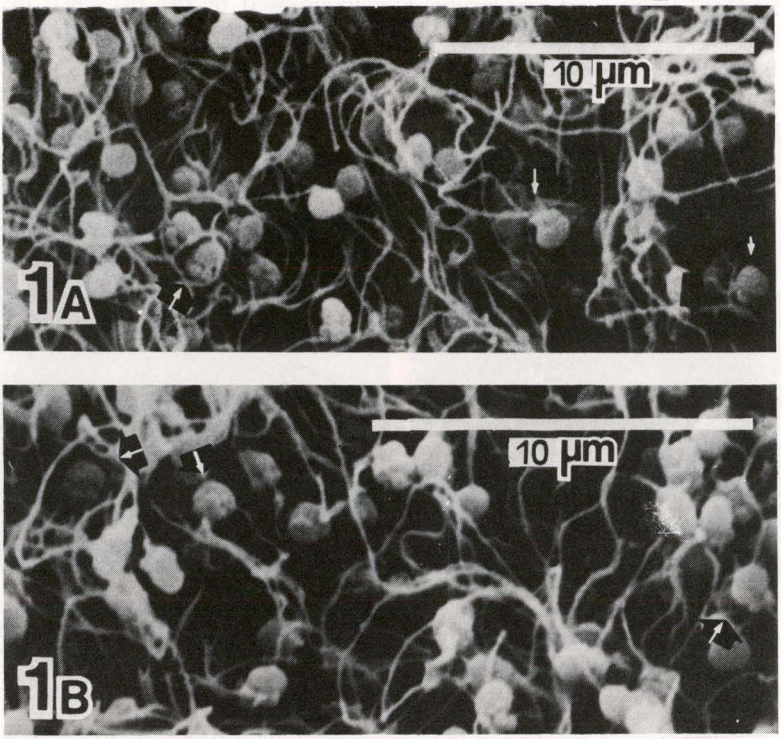


Fig. 1. (a, b) SEM view of the **M. curema** semen; (→) spermatozoon.

Flagellum. The flagellum extends from the part of the spermatozoon tail situated in the transition region below the lamella to the terminal portion of the sperm cell (Figs 2a,b; 6). In the apical region of the flagellum, near the transition region, it was observed that configuration of the axial filaments is of the 9 + 0 type (Figs 2a,b), but the central axial filaments are present along other areas of the axoneme in the 9 + 2 disposition (Figs 6, 7).

Transverse sections of the apical region of the axoneme evidenced Y-shaped bridges linking the peripheral axial filaments to the flagellum wall (Fig. 5).

It was noticed that in over 50% of the spermatozoa analysed that the peripheral axial filaments were electron-dense on A-tubules numbers 1, 2, 5 and 6 (Fig. 7).

DISCUSSION

The lack of an acrosome in spermatozoa of the *Mugil curema* species is a feature common to the Teleostei (NICANDER, 1970; MATTEI, 1970). It is possible that the loss of this structure ocurred during the evolutionary process

(AFZELIUS, 1978), and was probably compensated by the presence of a micropyle in the eggs (PASTEELS, 1965a,b; GINSBURG, 1968) which facilitates the penetration of the male gamete making fecundation possible.

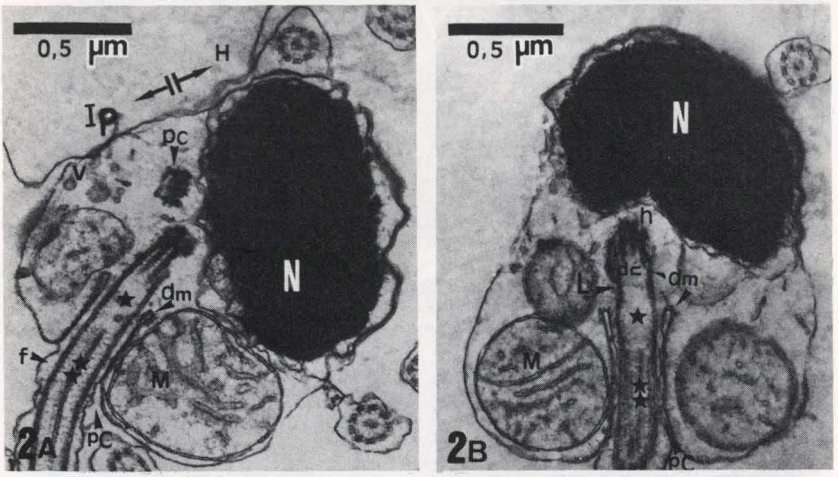
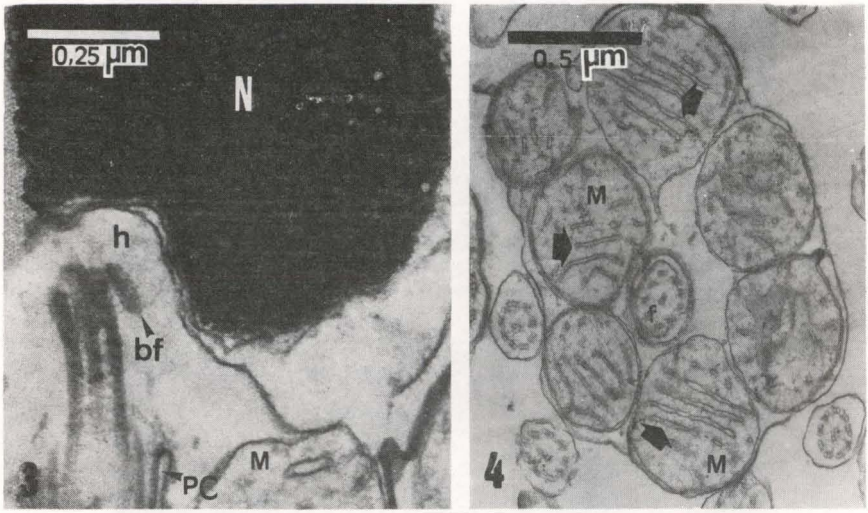


Fig. 2. (a, b) TEM longitudinal section of **M. curema** spermatozoon; (h) head; (Ip) midpiece; (f) flagellum; (N) nucleus; (pc) proximal centriole; (dc) distal centriole; (dm) dense material; (M) mitochondria; (pC) plasmic canal; (V) vesicle; (L) lamella; (*) microtubules of the 9+0 type; (**) microtubules of the 9+2 type; (h) hilus.

The basic configuration of the *M. curema* Valenciennes, 1836 spermatozoa consisting of head, midpiece with most of the functional structures and a single flagellum, is similar to that of other Teleostei. However, the individual analysis of each of these parts reveals that the spermatozoa may vary widely in form and structure.

The sperm head of the *M. curema* is rounded as in *M. liza* and *M. planatus* Günther, 1880 (EIRA-STOFELLA & GREMSKI, 1991), *Liza aurata* Risso, 1810 (BRUSLE, 1981), and *L. dumerili* (VAN DER HORST & CROSS, 1978), as well as in some representatives of other fish families (POIRIER & NICHOLSON, 1982; MATTEI & MATTEI, 1984). However, in many Teleostei, the head of the spermatozoon is extremely long and cone-shaped (STANLEY, 1969; VAN DEURS & LASTEIN, 1973; GRIER, 1975; MATTEI & MATTEI, 1978; MATTEI *et al.*, 1989). In terms of the diameter of the spermatozoon head, which in this case also comprises part of the midpiece, it is about 1.56 μm in *M. curema* and therefore similar to *M. liza* and *M. planatus*, which both measuring 1.50 μm (EIRA-STOFELLA & GREMSKI, 1991), but much smaller than in *Liza dumerili*, which has a diameter of 2.4 μm (VAN DER HORST & CROSS, 1978), or other Teleostei in general. According to GINSBURG (1978), the spermatozoon head rounded in the Teleostei and has a diameter of 2-3 μm.

The cellular and nuclear membrane of the Teleostei spermatozoa are generally marked by the presence of a double wall and an undulant contour



Figs 3-4. **M. curema**. (3) Longitudinal section of the head spermatozoon. (N) Nucleus; (h) nuclear hilus; (bf) basal foot; (pC) plasmic canal; (M) mitochondria. (4) Transversal section of the spermatozoon showing the large number of mitochondria (M) and the flagellum; (→) plate-like mitochondrial cristae.

(BRUSLE, 1981; POIRIER & NICHOLSON, 1982; EIRAS-STOFELLA & GREMSKI, 1991), similar to *Mugil curema*.

In *M. liza* and *M. platanus* the nucleus, which is located in the apical region of the spermatozoon head, has a chromatin that becomes gradually more compact with gonad maturation. The direction of this condesation was observed (EIRAS-STOFELLA & GREMSKI, 1991). In *M. curema* the nucleus is also placed in the apical position, but the chromatin granules are so close to each other that they form an extremely electron-dense compact mass, which does not allow determination of the direction of chromatin maturation. Therefore, it is possible that all the semen presently analysed was at a more advanced stage of maturation than in the other Mugilidae studied. The nucleus in *L. dumerili* (VAN DER HORST & CROSS, 1978) and *L. aurata* (BRUSLE, 1981) presented several chromatin granules, but these were divided into less clearly marked groups than in *m. curema*.

The nucleus is generally kidney-shaped in Teleostei with a rounded spermatozoon head, and the curved apical region of the nucleus is separated from the apex of the sperm cell only by the nuclear membrane. A more or less marked groove was found in the basal nuclear part that forms the nuclear hilus next to the point of implantation of the flagellum. This is the same type of configuration found in the nucleus of the *M. curema*, in other Mugilidae (VAN DER HORST & CROSS, 1978; BRUSLE, 1981; EIRAS-STOFELLA & GREMSKI, 1991) and in species belonging to other fish families (MATTEI, 1970).

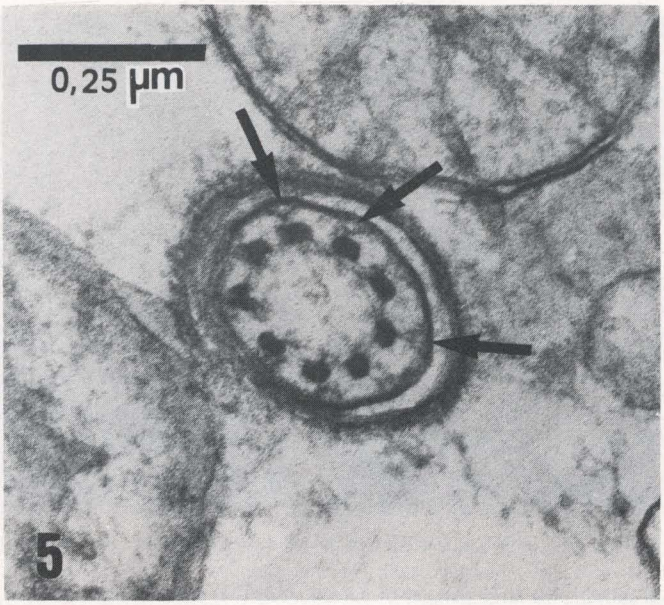


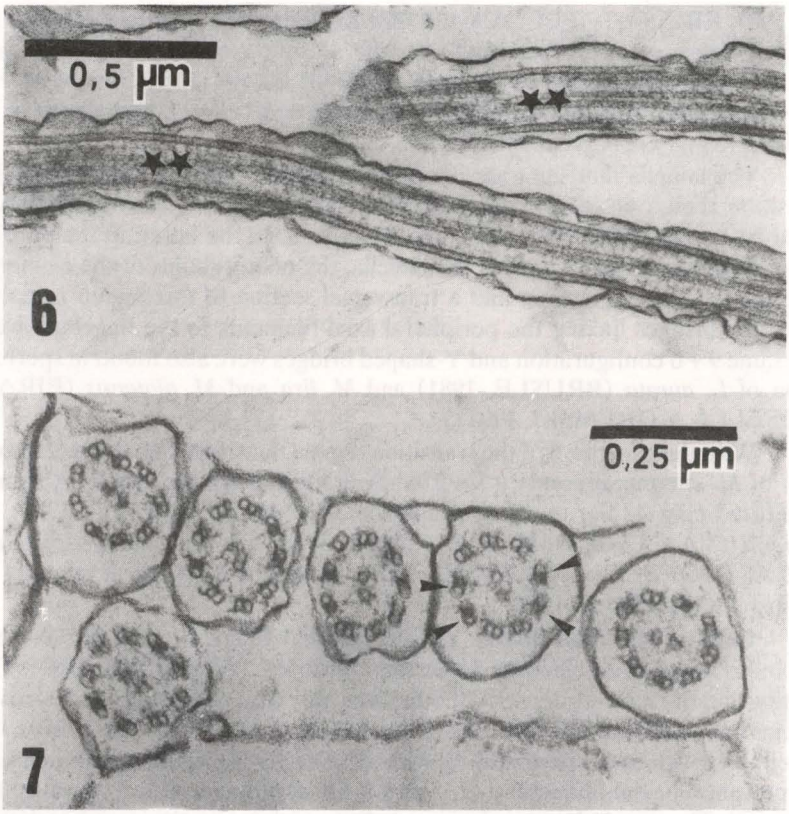
Fig. 5. Transition region of the spermatozoon flagellum of **M. curema** indicating the Y-shaped bridges (→).

The diameter of the nucleus of *M. curema* spermatozoa (1.08μm) is smaller than that of the *L. aurata* which is about 1.5μm (BRUSLE, 1981).

The midpiece of the sperm cell is often well developed in Teleostei (VAN DEURS & LASTEIN, 1973; POIRIER & NICHOLSON, 1982) and may even be divided into sections (MATTEI & MATTEI, 1978). However, analysis of spermatozoa of fishes belonging to the family Mugilidae (VAN DER HORST & CROSS, 1978; BRUSLE, 1981; EIRAS-STOFELLA & GREMSKI, 1991) in general, and especially to the *Mugil curema* species, revealed that the midpiece is discrete in these fishes. GRIER *et al.* (1978) named this region of the male gamete of Goodeidae fishes as a pseudo-midpiece.

The proximal centriole of *M. curema* spermatozoon is similar to that of *L. dumerili* (VAN DER HORST & CROSS, 1978), *M. liza* and *M. platanus* (EIRAS-STOFELLA & GREMSKI, 1991) and to *L. aurata* (BRUSLE, 1981), because of its oblique position in relation to the distal centriole.

The midpiece of many Teleostei contains three to four mitochondria, but this number may vary considerably among different species, although not with the same one (GINSBURG, 1968). About four mitochondria were found in each cell of the spermatozoon already studied in the family Mugilidae (VAN DER HORST & CROSS, 1978; BRUSLE, 1981; EIRAS-STOFELLA & GREMSKI, 1991). However, nine to 10 mitochondria were observed in *M. curema* each with a diameter of 0.59μm. The diameter of mitochondria in *M. curema* is similar to that of *L. aurata* (BRUSLE, 1981), which is 0.55-0.66μm, but differs from that



Figs 6-7. **M. curema**. (6) Longitudinal section of the flagellum in the semen; (→) microtubules of the 9+2 type. (7) Transverse section of the flagellum of the spermatozoon where the electron density of A-tubules 1, 2, 5 and 6 can be observed (→).

of *M. platanus* and *M. liza* (EIRAS-STOFELLA & GREMSKI, 1991) which is smaller (0.35μm). Two types different mitochondrial cristae were found in *M. liza* and *M. platanus* (EIRAS-STOFELLA & GREMSKI, 1991), *L. aurata* (BRUSLE, 1981) and *L. dumerili* (VAN DER HORST & CROSS, 1978). The type of cristae known as plate is common to all of these species, but it was the only one found in the mitochondria of *M. curema*.

Several vesicles were found in the sperm cells of *M. liza* and *M. platanus* (EIRAS-STOFELLA & GREMSKI, 1991), but these structures were rare in *M. curema*, and absent in *L. aurata* (BRUSLE, 1981) and *L. dumerili* (VAN DER HORST & CROSS, 1978). The basal foot, which expands from the distal centriole towards the nucleus in the midpiece of *M. curema* spermatozoon, was found in *M. platanus* and *M. liza* (EIRAS-STOFELLA & GREMSKI, 1991).

The plasmic canal is a characteristic structure of all male gametes of Mugilidae fish already described (VAN DER HORST & CROSS, 1978; BRUS-

LE, 1981; EIRAS-STOFELLA & GREMSKI, 1991), *M. curema* included. The layer of dense material that reinforces the plasmic canal is common to and similar between *M. curema* and *L. aurata* (BRUSLE, 1981), denser in the *M. liza* and *M. platanus* (EIRAS-STOFELLA & GREMSKI, 1991), and absent in *L. dumerili* (VAN DER HORST & CROSS, 1978).

The lamella that separates the distal centriole from the flagellum in the transition region of sperm cells is common in *M. liza*, *M. platanus* (EIRAS-STOFELLA & GREMSKI, 1991) and *M. curema*. In the latter, in the portion of the flagellum situated next to the lamella, the microtubules of the axoneme have the 9+0 configuration and a transversal section of this region revealed Y-shaped bridges linking the peripheral axial filaments to the flagellum wall. The same 9+0 configuration and Y-shaped bridges were also found in spermatozoa of *L. aurata* (BRUSLE, 1981) and *M. liza* and *M. platanus* (EIRAS-STOFELLA & GREMSKI, 1991).

With the exception of the transition region, the single flagellum of sperm cells of *M. curema* presents a 9+2 configuration along the whole axoneme, therefore being similar to the spermatozoa of *M. aurata* (BRUSLE, 1981), *L. dumerili* (VAN DER HORST & CROSS, 1978), *M. liza* and *M. platanus* (EIRAS-STOFELLS & GREMSKI, 1991), as well as in the great majority of Teleostei (AFZELIUS, 1981).

Each of the nine double peripheral axial filaments contains A- and B-tubules being more electron-dense than B-tubules (AFZELIUS, 1981). The distribution of electron-dense A-tubules was observed in most of the Teleostei species analysed (AFZELIUS, 1981). According to MATTEI *et al.* (1979), the flagellum of Teleostei spermatozoa, with the A-tubules more electron-dense in the peripheral axial filaments 1, 2, 5 and 6, the asymmetry of the sperm cell is indicated. The A-tubules ara also more electron-dense in the peripheral axial filaments 1, 2, 5 and 6 in *M. liza* and *M. platanus* spermatozoa (EIRAS-STOFELLA & GREMSKI, 1991), similar to the species which is the object of the present study. Therefore, this microtubule configuration of *M. curema* reinforces the asymmetry of its spermatozoa if one considers that the distal centriole forms an oblique angle with the proximal centriole.

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