

Spermiogenesis and spermatozoa ultrastructure in the Serrasalminae (Ostariophysi: Characiformes) with further evidence on the relationships of the piranhas and pacus

Priscila Gusmão-Pompiani¹, Luiz R. Malabarba², Claudio Oliveira³
and Irani Quagio-Grassiotto³

Spermatozoa and spermiogenesis ultrastructure were studied in the serrasalmine species *Piaractus mesopotamicus*, *Mylossoma duriventre*, *Serrasalmus maculatus*, and *Metynnis mola* and two distinct patterns may be recognized: the first common to *Mylossoma*, *Serrasalmus* and *Metynnis*, and the other, characteristic of *Piaractus*. The latter pattern is more similar to the conditions found in *Salminus* and *Brycon*. On the other hand, serrasalmine spermatozoa also share characteristics with the spermatozoa of species of the superfamily Anostomoidea. The phylogenetic significance of these characters is discussed.

A ultraestrutura dos espermatozoides e espermiogênese foram estudadas nas espécies *Piaractus mesopotamicus*, *Mylossoma duriventre*, *Serrasalmus maculatus* e *Metynnis mola* de Serrasalminae, sendo dois padrões distintos reconhecidos: um comum a *Mylossoma*, *Serrasalmus* e *Metynnis*, e o segundo característico de *Piaractus*. Este último é mais similar ao observado em *Salminus* e *Brycon*. Por outro lado, o espermatozoide dos serrasalmíneos compartilha algumas características com espécies da superfamília Anostomoidea. O significado filogenético destes caracteres é discutido.

Key words: Characid, Spermatid, Spermatozoa, Phylogeny, Morphology.

Introduction

The Serrasalminae consists of 81 species and 16 genera (Eschmeyer & Fong, 2009), including the carnivorous piranhas, and the seed and fruit eating tambaquis and pacus. These fishes are strictly Neotropical, and occur in all freshwater biotopes, except in very narrow forest brooks and benthic areas of deep rivers. Serrasalmines have a deep, laterally compressed body, with a series of mid-ventral abdominal spines and, except for *Colossoma*, *Piaractus* and *Mylossoma*, they have an anteriorly directed spine just anterior to the dorsal fin. Particularities of the buccal apparatus of these species are associated with feedings habits, including carnivory, frugivory and lepidophagy (Géry, 1977; Jégu, 2003).

Monophyly of the Serrasalminae has been well-established (Machado-Allison, 1982), but the group has been

alternatively treated as a separate family or as a subfamily of the Characidae. We follow Buckup (1998) and Jégu (2003) in placing the piranhas as a subfamily of the Characidae.

Based on dental morphology, Eigenmann (1915) initially divided serrasalmines in two subfamilies, Serrasalminae and Myleinae, including respectively the carnivorous piranhas, and the seed and fruit eating tambaquis and pacus. Machado-Allison (1982) using cladistic methods, defined the Serrasalminae as monophyletic based on morphological characters, and divided it in two clades, one including all the pacus and most of the Myleinae of Eigenmann, with the genera *Colossoma*, *Piaractus* and *Mylossoma* in a relatively derived position, and the other clade including all the piranhas (the Serrasalminae of Eigenmann).

Recent morphological and molecular phylogenetic studies have changed previous concepts that used to split

¹Universidade Estadual do Mato Grosso do Sul, Unidade de Ensino de Coxim. Rua Pereira Gomes, 355, 79400-000 Coxim, Mato Grosso do Sul, Brazil. priscila_pompiani@yahoo.com.br

²Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, 91501-970 Porto Alegre, Rio Grande do Sul, Brazil. malabarba@ufrgs.br

³Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, CP 510, 18.618-000 Botucatu, São Paulo, Brazil. claudio@ibb.unesp.br (CO), iraniqg@ibb.unesp.br (IQG)

serrasalmines into carnivorous *versus* herbivorous groups. Despite differences in the relative phylogenetic position of each genus, recent hypotheses are congruent in placing *Piaractus*, *Mylossoma* and *Colossoma* in a relative basal position among serrasalmines, and the genera *Pygocentrus*, *Serrasalmus*, *Pristobrycon*, and *Pygopristis* as the most derived taxa in a larger clade containing the remaining serrasalmine genera.

Ortí *et al.* (1996, 2008), using molecular data, found three major clades within serrasalmines: (1) a basal clade formed by *Colossoma*, *Mylossoma*, and *Piaractus*; (2) the “*Myleus*” clade, constituted by *Myleus*, *Mylesinus*, *Tometes*, and *Ossubtus*; and (3) the “piranha” clade, containing *Serrasalmus*, *Pygocentrus*, *Pygopristis*, *Pristobrycon*, *Catoprion*, and *Metynnis*. The genus *Acnodon* was placed as the sister taxon of clade (2+3).

The phylogenetic study of Calcagnotto *et al.* (2005), also based on molecular data and including a larger number of characiform taxa, also supports the Serrasalminae as monophyletic, but as a lineage separated from the Characidae and sister taxa of the Anostomoidea. Although Calcagnotto *et al.* (2005) used a smaller number of genera (8) than Ortí *et al.* (1996, 2008), the ingroup relationships found by the former authors are very similar, except for splitting the basal “pacus” clade, by positioning *Colossoma* and *Mylossoma* as a sister clade to *Metynnis* + (*Pygocentrus* + *Serrasalmus*), and *Piaractus* as the most basal genus. The relationships of serrasalmines and the Anostomoidea, however, are not supported by the great morphological diversity shown by those taxa.

Spermatozoa ultrastructure has been considered as potentially informative to solve questions of phylogenetic relationships among distinct groups of fish (Jamieson, 1991; Mattei, 1991). Spermiogenesis and spermatozoa types, organell distribution and other particularities of the spermatid structure, have been found to be highly conservative in a given lineage at family or subfamily level, making them useful for phylogenetic analysis (Baccetti *et al.*, 1984; Jamieson, 1991; Mattei, 1991; Burns *et al.*, 1998; Quagio-Grassiotto *et al.*, 2003). Regarding the Characiformes, and particularly the Characidae, the available information is still scarce considering the large diversity of the group (Jamieson, 1991; Matos *et al.*, 1993, 1998, 2000; Burns *et al.*, 1998; Magalhães, 1998; Pecio & Rafinski, 1999; Romagosa *et al.*, 1999; Aires *et al.*, 2000; Zaiden, 2000; Andrade *et al.*, 2001; Quagio-Grassiotto *et al.*, 2001a, 2003; Amaral, 2003; Cruz-Landim *et al.*, 2003; Pecio, 2003; Azevedo, 2004; Burns & Weitzman, 2005; Pecio *et al.*, 2005, 2007; Weitzman *et al.*, 2005; Veríssimo-Silveira *et al.*, 2006).

Herein, spermiogenesis and spermatozoa of representatives of the three taxa of serrasalmines proposed by Ortí *et al.* (1996, 2008) are described and compared with representatives of other characiform lineages. Phylogenetic implications and possible relationships of serrasalmines based characters derived from spermiogenesis and spermatozoa structure are discussed.

Material and Methods

Adult males of *Piaractus mesopotamicus* (LBP 6757), *Mylossoma duriventre* (LBP 5148), *Serrasalmus maculatus* (LBP 6757), and *Metynnis mola* (LBP 3216), were collected in the Taquari River, Coxim, Mato Grosso do Sul, Brazil (18°25'42.5"S 54°50'02.8"W). The fish were identified and kept in the fish collection of Laboratório de Biologia e Genética de Peixes (LBP), Departamento de Morfologia, Instituto de Biociências, UNESP, Botucatu, SP, Brazil.

Gonad fragments were fixed overnight in 2% glutaraldehyde and 4% paraformaldehyde in 0.1 M Sorensen phosphate buffer, pH 7.4. The material was post-fixed in the dark for 2 hours in 1% osmium tetroxide in the same buffer, stained in block with aqueous solution of 5% uranyl acetate for two hours, dehydrated in acetone, embedded in araldite, and sectioned and stained with a saturated solution of uranyl acetate in 50% ethanol and lead citrate. Electron micrographs were obtained using a Phillips - CM 100 transmission electron microscope.

Results

The spermiogenesis process is quite similar in all species of serrasalmines analyzed and is herein represented by *Serrasalmus maculatus*.

Spermiogenesis of *Serrasalmus maculatus*

In the early spermatids, the cytoplasm symmetrically encircles the nucleus, which displays diffuse homogenous chromatin, and has a circular outline. The centriolar complex lies lateral to the nucleus, and is anchored to the plasma membrane. The proximal centriole is anterior and perpendicular relative to the distal centriole. The centrioles are covered with homogeneous and electron dense material and fastened to each other by microfibrils. The electron dense material that covers the centrioles is initially diffuse and granular. The distal centriole differentiates into the basal body and forms the flagellum. The centriolar complex moves toward the nucleus, carrying along the plasma membrane and the initial segment of the flagellum, which invaginates. The cytoplasmic canal, a space between the flagellum and the plasma membrane, is then formed (Fig. 1A and B). A depression is formed in the nuclear outline at the level of the centriolar complex. The nucleus rotates during spermiogenesis, and the centriolar complex penetrates the newly formed depression (Fig. 1B, C and D). Most of the cytoplasm concentrates in the region surrounding the cytoplasmic canal, forming the midpiece which contains elongate mitochondria. Abundant vesicles are present in the midpiece (Fig. 1E, F and G).

The chromatin condenses, and progressively forms thicker fibers. In the final spermatids, the nucleus contains highly condensed filamentous floccus of chromatin (Fig. 1C, D and E). The flagellum contains a classic (9+2) axoneme, is surrounded by the flagellar membrane, and forms flagellar fins (Fig. 1H, I and J).

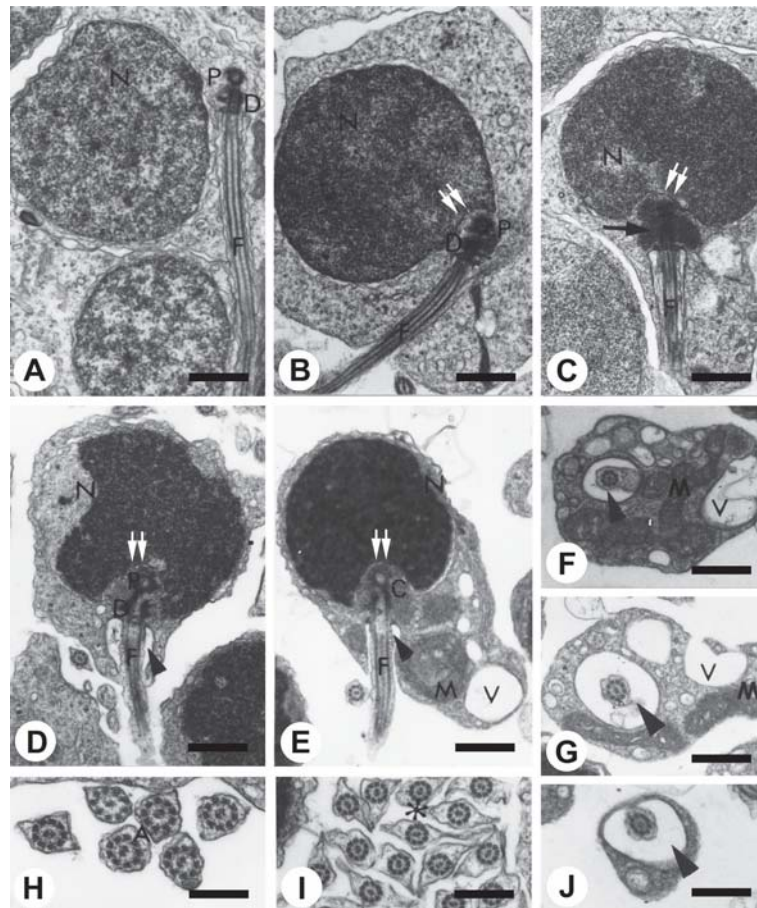


Fig. 1. *Serrasalmus maculatus* spermiogenesis. A to C - Longitudinal sections of early spermatids during the nuclear rotation process. D and E - Longitudinal sections of early spermatids during the chromatin condensation process. F, G and J - Midpiece cross sections. H and I - Flagella in cross sections. Scale bars: A to C = 1 μm ; D = 0.85 μm ; E = 0.9 μm ; F = 0.9 μm ; G = 0.75 μm ; H = 0.04 μm ; I = 0.75 μm ; J = 0.6 μm . A: axoneme; C: centriole complex; D: distal centriole; F: flagellum; M: mitochondria; N: nucleus; P: proximal centriole; V: vesicle; Asterisk: fins; Arrow: electron dense material; Arrowhead: cytoplasmic canal; Double arrow: nuclear fossa.

Spermatozoa of *Piaractus mesopotamicus*

In the spermatozoa of *P. mesopotamicus*, the conical nucleus (1.8 μm in length and 1.4 μm in width) contains large flocus of highly condensed chromatin, and is surrounded by a narrow strip of cytoplasm with no organelles (Fig. 2A). In the nuclear outline, that faces the midpiece, there is a median deep depression in a slight eccentric position, the nuclear fossa (Fig. 2A). The centrioles are fastened to one another and to the nuclear envelope, at the nuclear fossa, by stabilization fibrils. The distal centriole is also fastened to the plasma membrane. The proximal centriole is anterior, and perpendicular to slightly oblique in relation to the distal centriole. The centriolar complex is inside the nuclear fossa. The distal centriole differentiates into the basal body and gives rise to the axoneme (Fig. 2B, C and D). The flagellum is slightly eccentric to the nuclear axis and the midpiece is slightly asymmetric. The midpiece, with 1.8 μm in length, has a short terminal cytoplasmic sleeve, contains the mitochondria and the cytoplasmic canal into which is the

initial segment of the flagellum (Fig. 2A and A-Inset). The mitochondria are long, probably single, and form a ring around the cytoplasmic canal (Fig. 2F and G). The flagellum contains the classic axoneme (9+2) and has two short flagellar fins (Fig. 2E and H).

Spermatozoa of *Serrasalmus maculatus*

In the spermatozoa of *S. maculatus* the spherical nucleus (with 1.9 μm in diameter) contains highly condensed flocculent chromatin interspersed by electron-lucent areas, and is surrounded by a narrow strip of cytoplasm with no organelles (Fig. 3A). In the nuclear outline, that faces the midpiece, there is a median deep depression in a slight eccentric position, the nuclear fossa (Fig. 3A). The centrioles are covered by electron dense material and fastened to one another and to the nuclear envelope at the nuclear fossa by stabilization fibrils. The distal centriole is also fastened to the plasma membrane. The proximal centriole is anterior, and perpendicular in relation to the distal centriole. The proximal centriole is inside the nuclear

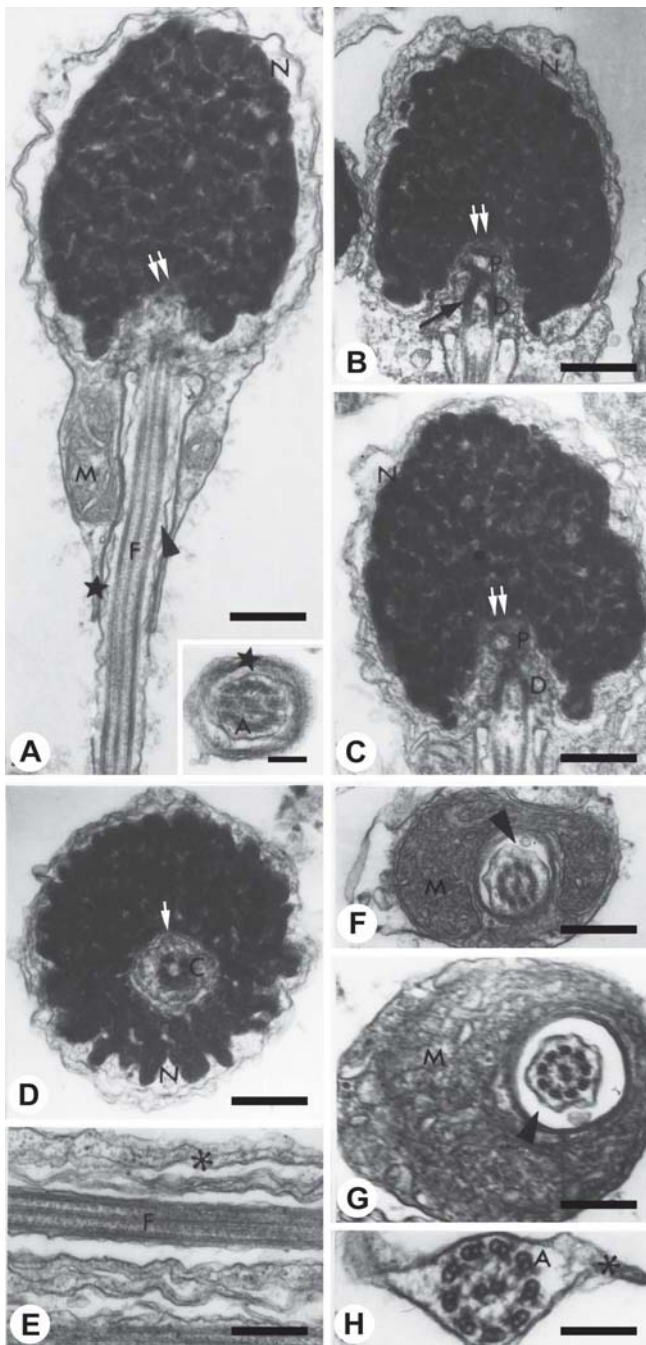


Fig. 2. *Piaraactus mesopotamicus* spermatozoa. A - Longitudinal section of a spermatozoon. A/Inset - Cross section of the midpiece at the cytoplasmic sleeve region. B and C - Longitudinal sections of the nucleus at the nuclear fossa region. D - Cross sections of the nucleus at the nuclear fossa region. E and F - Cross sections in different levels of the midpiece. G and H - Longitudinal and cross sections of the flagellum. Scale bars, A = 0.45 μm ; A/inset = 0,15 μm ; B and C = 0.4 μm ; D = 0.35 μm ; E = 0.3 μm ; F = 0.25 μm ; G = 0.3 μm ; H = 0.15 μm . A: axoneme; C: centriole; D: distal centriole; F: flagellum; M: mitochondria; N: nucleus; P: proximal centriole; Asterisk: fins; Arrow: electron dense material; Arrowhead: cytoplasmic canal; Double arrow: nuclear fossa; Star: cytoplasmic sleeve.

fossa while the distal centriole remains outside. The distal centriole differentiated into the basal body forms the axoneme (Fig. 3A, A-Insert and B). The flagellum is slightly eccentric to the nuclear axis (Fig. 3A). The midpiece has 3.0 μm in length, contains the mitochondria, large vesicles and the cytoplasmic canal into which is the initial segment of the flagellum (Fig. 3A). The midpiece is strongly asymmetric due the unequal distribution of the mitochondria and the vesicles. The mitochondria are very long, can be ramified and are found close to the nucleus and accumulated in one of the sides of the midpiece. The large vesicles are found at the same side the midpiece in which is also the mitochondria (Fig. 3D-J). The flagellum contains a classic axoneme (9+2), a vesicular compartment alongside the initial segment and has two long flagellar fins (Fig. 3K and L).

Spermatozoa of *Mylossoma duriventre*

In spermatozoa of *M. duriventre*, the spherical nucleus (with 1.9 μm in diameter) contains highly condensed flocculent chromatin interspersed by electron-lucent areas, and is surrounded by a narrow strip of cytoplasm with no organelles (Fig. 4A). In the nuclear outline, that faces the midpiece, there is a median deep depression in a slight eccentric position, the nuclear fossa (Fig. 4A, B and C). The centrioles are covered by electron dense material and fastened to one another and to the nuclear envelope at the nuclear fossa by stabilization fibrils. The distal centriole is also fastened to the plasma membrane. The proximal centriole is anterior, and perpendicular in relation to the distal centriole. The proximal centriole is inside the nuclear fossa while the distal centriole remains outside. The distal centriole differentiated into the basal body forms the axoneme (Fig. 4B and C). The flagellum is slightly eccentric to the nuclear axis and the midpiece is slightly asymmetric. The midpiece, with 1.4 in length, has a very short terminal cytoplasmic sleeve, contains the mitochondria, abundant vesicles and the cytoplasmic canal into which is the initial segment of the flagellum (Fig. 4A, D). The midpiece is asymmetric due the unequal distribution of the mitochondria and the vesicles. The mitochondria are very long, can be ramified and are found close to the nucleus and accumulated in one of the sides of the midpiece. The large vesicles are found at the same side the midpiece in which is also the mitochondria (Fig. 4G-H). The flagellum contains a classic axoneme (9+2) and has two or occasionally tree long flagellar fins (Fig. 4L, J and K).

Spermatozoa of *Metynniss mola*

In the spermatozoa of *M. mola*, the spherical nucleus (with 1.9 μm in diameter) contains floccus of highly condensed chromatin, interspersed by electron lucent areas, and surrounded by a narrow strip of cytoplasm with no organelles (Fig. 5A). In the nuclear outline, that faces the midpiece, there is a median deep depression in a slight eccentric position, the nuclear fossa (Fig. 5B). The centrioles are covered by electron dense material and fastened to one another and to the nuclear envelope, at the nuclear fossa, by stabilization fibrils. The

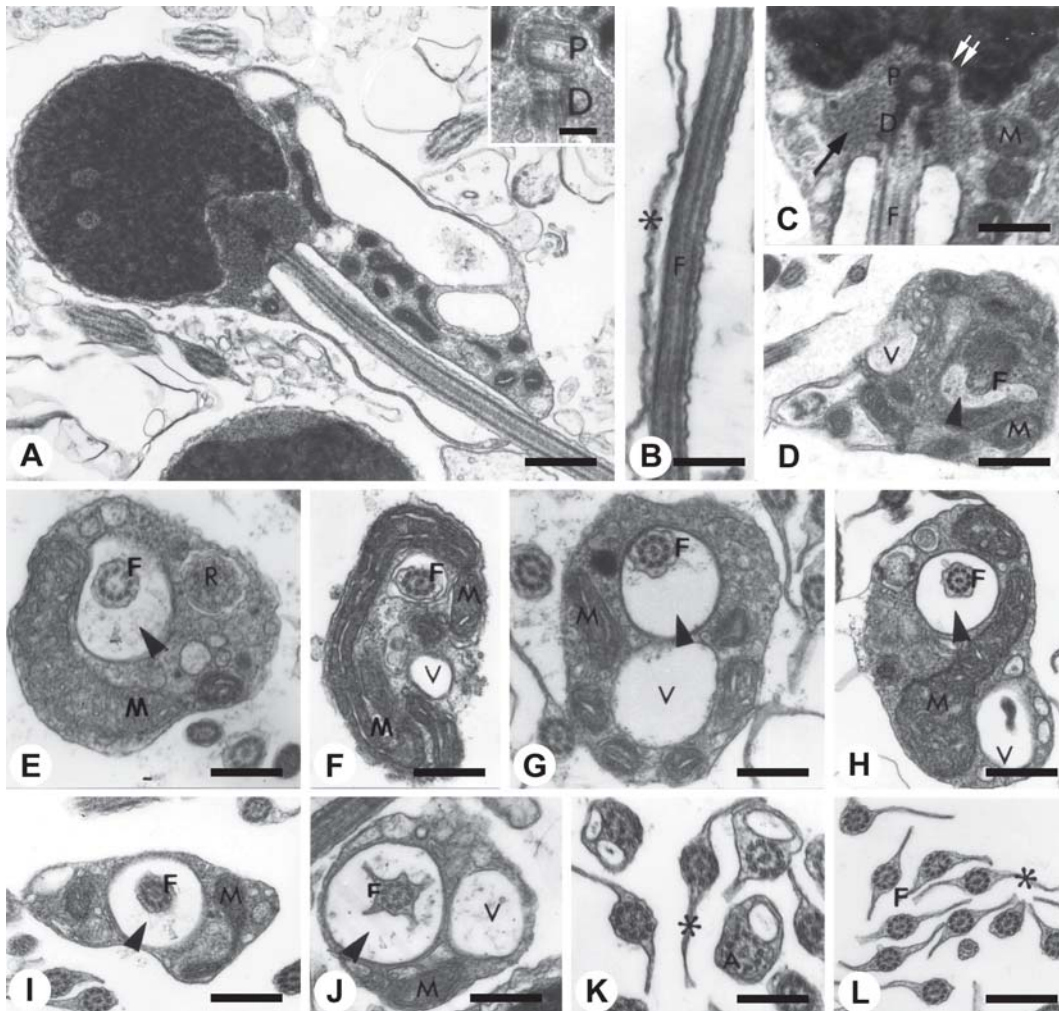


Fig. 3. *Serrasalmus maculatus* spermatozoa. A - Spermatozoa in longitudinal section. B - Flagellum in longitudinal section. C - Longitudinal section of the nucleus at the nuclear fossa region. D to J - Cross sections in different levels of midpiece. K and L - Flagella in cross sections. Note the vesicles in the initial segment of the flagella. Scale bars A = 0.5 μ m; A/Inset = 0.2 μ m; B-C = 0.45 μ m; D = 0.7 μ m; E and F = 0.4 μ m; G = 0.5 μ m; H and I = 0.55 μ m; J = 0.4 μ m; K = 0.35 μ m; L = 0.6 μ m. A: axoneme; C: centriole; D: distal centriole; F: flagellum; M: mitochondria; N: nucleus; P: proximal centriole; V: vesicle; Asterisk: fins; Arrow: electron dense material; Arrowhead: cytoplasmic canal; Double arrow: nuclear fossa; Star: cytoplasmic sleeve.

distal centriole is also fastened to the plasma membrane. The proximal centriole is anterior, and perpendicular to slightly oblique in relation to the distal centriole. The proximal centriole and the initial tip of the distal centriole are inside the nuclear fossa. The distal centriole differentiated into the basal body forms the axoneme (Fig. 5C and D). The flagellum is slightly eccentric to the nuclear axis and the midpiece is slightly asymmetric (Fig. 5A). The midpiece, with 2.0 μ m in length, has a very short terminal cytoplasmic sleeve, contains the mitochondria, abundant vesicles and the cytoplasmic canal into which is the initial segment of the flagellum. The mitochondria are very long, can be ramified and are found close to the nucleus and accumulated in one of the sides of the midpiece. The vesicles are found at the same side the midpiece in which is the mitochondria (Fig. 5C-G).

The flagellum contains the classic axoneme (9+2). A tubule-vesicular compartment runs alongside of the flagellum, decreases gradually and disappears in the flagellar terminal end (Fig. 5I-L).

Discussion

Species of the Serrasalminae share the dominant type of spermatozoa (type I) found among most actinopterygian and external fertilizing Characiformes. The cellular process that culminates with the formation of a type I (*sensu* Mattei, 1970) sperm is very similar in all the species analyzed. This classification is based on whether nuclear rotation, in relation to the flagellar axis, occurs or not during spermiogenesis. During that process the flagellum generally develops lateral

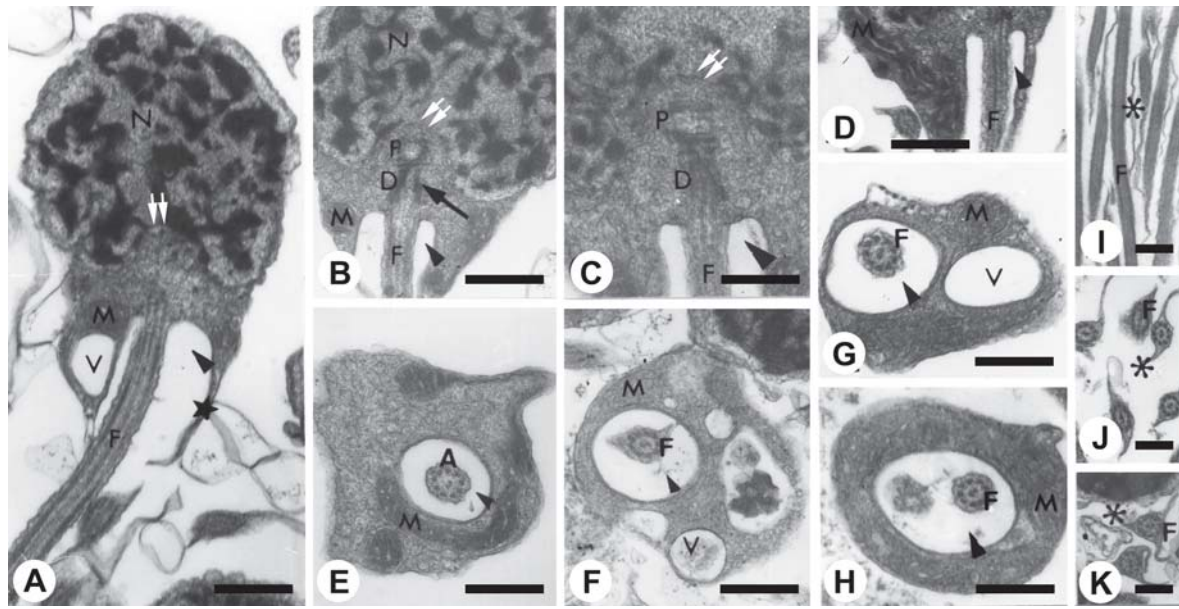


Fig. 4. *Mylossoma duriventre* spermatozoa. A - Spermatozoa in longitudinal section. B and C - Longitudinal sections of the nuclear fossa region. D - Midpiece in longitudinal section. E to H - Midpiece in cross sections. I - Flagellum in longitudinal section. J and K - Flagella in cross sections. Scale bars, A = 0.55 μm ; B = 0.6 μm ; C = 0.35 μm ; D = 0.6 μm ; E - F = 0.45 μm ; G = 0.35 μm ; H = 0.4 μm ; I = 0.5 μm ; J = 0.3 μm ; K = 0.25 μm . A: axoneme; D: distal centriole; F: flagellum; M: mitochondria; N: nucleus; P: proximal centriole; V: vesicle; Asterisk: fins; Arrow: electron dense material; Arrowhead: cytoplasmic canal; Double arrow: nuclear fossa; Star: cytoplasmic sleeve.

to the nucleus in the early spermatids. When nuclear rotation occurs, the flagellum becomes perpendicular and medial to the nucleus, and the resulting spermatozoa are of the type I. If no nuclear rotation occurs, the flagellum remains parallel to the nucleus, and spermatozoa are of the type II. Nuclear rotation, however, may be incomplete. In this case, the flagellum is eccentric to the nuclear axis, and the spermatozoa are of an intermediate type between types I and II (Mattei, 1970). Among the external fertilizing Characiformes, spermatozoa of the type I is the dominant type (Jamieson, 1991; Matos *et al.*, 1993, 1998; Burns *et al.*, 1998; Magalhães, 1998; Romagosa *et al.*, 1999; Aires *et al.*, 2000; Zaiden, 2000; Andrade *et al.*, 2001; Quagio-Grassiotto *et al.*, 2001a, 2003; Amaral, 2003; Cruz-Landim *et al.*, 2003; Pecio, 2003; Azevedo, 2004; Burns & Weitzman, 2005; Weitzman *et al.*, 2005; Veríssimo-Silveira *et al.*, 2006; Oliveira, 2007; Pecio *et al.*, 2007; Veríssimo-Silveira, 2007).

The spermiatic ultrastructural characteristics of the species of Serrasalminae are quite homogeneous, showing a conservative spermiatic structure among the members of this lineage, corroborating previous propositions of serrasalmine monophyly. They share the type of chromatin condensation in floccus, the round shape of the nucleus, the length of the midpiece, and the long mitochondria found in the midpiece close to the nucleus and separated of the flagellum by the cytoplasmic canal.

Chromatin condensation occurs in the nucleus, during spermiogenesis. Different kinds of nuclear protein that are

associated with the DNA (Saperas *et al.*, 1993) confer different aspects to the spermatozoa nucleus. In the serrasalmine spermatozoa (Matos *et al.*, 1993; Cruz-Landim *et al.*, 2003; current paper) the nucleus is occupied by large floccus of fibrous chromatin. Among the Characiformes this kind of chromatin condensation has been described for *Citharinus* sp., Citharinidae (Mattei *et al.*, 1995), in *Leporinus friderici*, *Leporinus macrocephalus*, *Leporinus lacustris*, and *Abramites* sp., Anostomidae (Matos *et al.*, 1999; Amaral, 2003; Veríssimo-Silveira, 2007, respectively), in *Prochilodus lineatus*, Prochilodontidae (Vicentini *et al.*, 2001; Veríssimo-Silveira, 2007), in *Rhaphiodon vulpinus* Cynodontidae (Veríssimo-Silveira, 2007), in the characid genera *Salminus* (Veríssimo-Silveira *et al.*, 2006) and *Brycon* (Aires *et al.*, 2000; Romagosa *et al.*, 1999; Zaiden, 2000; Veríssimo-Silveira *et al.*, 2006), and in *Alestes dentex*, a relatively basal genus within the Alestidae (Shahin, 2006). Interestingly, in *Micralestes* sp. and *Phenacogrammus interruptus*, two derived taxa within the family Alestidae (see Zanata & Vari, 2005), the nucleus contain highly condensed, homogeneous granular chromatin (Veríssimo-Silveira, 2007), that may indicate later modifications of the chromatin in the evolution of derived taxa in Alestidae. Flocculent chromatin is also found in some inseminating species of the subfamily Stevardiinae (Pecio & Rafinski, 1999; Burns *et al.*, 1998; Burns & Weitzman, 2005; Pecio *et al.*, 2007).

The flocculent chromatin observed in serrasalmines, *Salminus* and *Brycon*, is clearly distinguishable from the highly

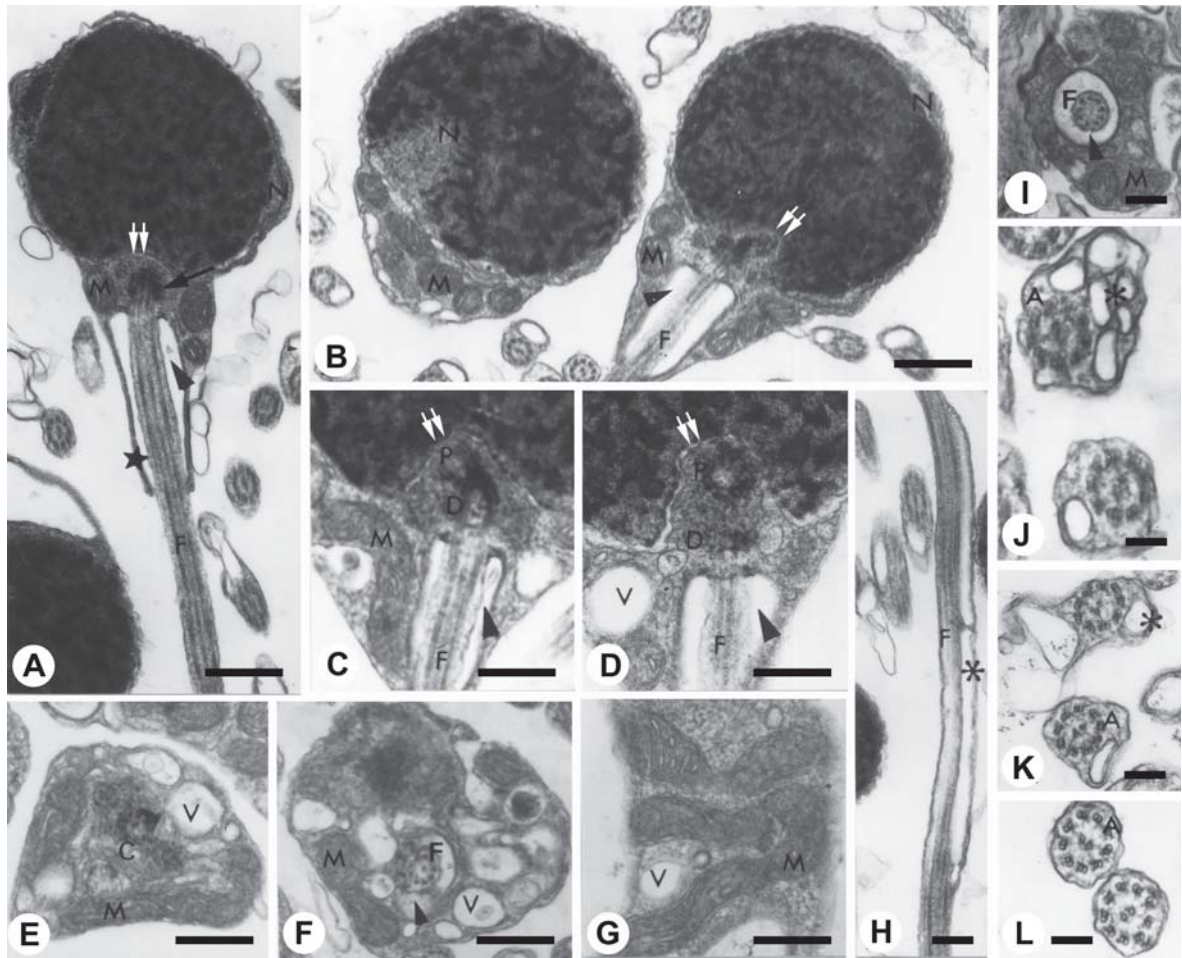


Fig. 5. *Metynnis mola* spermatozoa. A and B - Spermatozoa in longitudinal sections. C and D - Longitudinal sections of the nuclear fossa region. E to H - Midpiece in cross sections. I - Flagellum in longitudinal section. J, K and L - Flagella in cross sections. Scale bars, A = 0.6 μm ; B = 0.55 μm ; C = 0.4 μm ; D = 0.35 μm ; E = 0.5 μm ; F = 0.4 μm ; G = 0.35 μm ; H = 0.25 μm ; I = 0.35 μm ; J - K = 0,15 μm ; L = 0.1 μm . A: axoneme; C: centriole; D: distal centriole; F: flagellum; M: mitochondria; N: nucleus; P: proximal centriole; V: vesicle; Asterisk: tubule-vesicular compartment; Arrow: electron dense material; Arrowhead: cytoplasmic canal; Double arrow: nuclear fossa; Star: cytoplasmic sleeve.

condensed granular chromatin observed in the remaining studied characids: *Paracheirodon innesi* (Jamieson, 1991; as *Hyphessobrycon innesi*), *Hollandichthys* (Azevedo, 2004), Glandulocaudinae (Burns *et al.*, 1998; Pecio & Rafinski, 1999; Pecio *et al.*, 2005; Burns & Weitzman, 2005; Cheirodontinae Burns *et al.*, 1998; Burns & Weitzman, 2005; Oliveira, 2007); and *Bryconadenos* (Weitzman *et al.*, 2005).

The African citharinids have been commonly considered in both morphological and molecular analyses as basal characiforms (Vari, 1979; Ortí & Meyer, 1997; Buckup, 1998; Calcagnotto *et al.*, 2005). The spermatid characteristics of chromatin condensation found in citharinids (Mattei *et al.*, 1995) is similar to that found in *Chanos chanos*, a gonorynchiform (Gwo *et al.*, 1995), sister group of the Otophysi, and in *Olivaichthys mesembrinus*, Diplomystidae (Quagio-Grassiotto *et al.*, 2001b), the most basal family in Siluriformes, and may represent a primitive state of the

character that remains in some representatives of characiforms, including serrasalmines, *Salminus*, *Brycon*, stevardiines, anostomids, prochilodontids, and cynodontids. Among non-characid characiforms for which information is available, the large floccus of fibrous chromatin is not present in any other family of the Suborder Characoidei (Matos *et al.*, 2000; Quagio-Grassiotto *et al.*, 2001a; Veríssimo-Silveira, 2007).

Calcagnotto *et al.* (2005) postulated serrasalmines as a family and sister group to a monophyletic clade composed by the Anostomidae, Chilodontidae, Prochilodontidae, Hemiodontidae, and Parodontidae. Interestingly, serrasalmines, anostomids and prochilodontids share the presence of large floccus of fibrous chromatin, although it is not found in other studied Anostomoidea (families Curimatidae - Matos *et al.*, 1998; Quagio-Grassiotto *et al.*, 2003; and Chilodontidae - Pecio, 2003). However, the possible

plesiomorphic nature of the chromatin condensation does not permit to consider the spermatozoa ultrastructure as a support for the hypothesis of a close relationship between anostomids and serrasalmines. In addition to the flocculent chromatin, the Anostomidae (Matos *et al.* 1999; Amaral, 2003; Veríssimo-Silveira, 2007) shares with Serrasalminae the presence of flagellar fins. Among the Anostomoidea the flagellar fins are also found in the Chilodontidae (Pecio, 2003; Veríssimo-Silveira, 2007).

The spermiatic characteristics of the serrasalmine genera herein analyzed show two distinct patterns: one common to *Mylossoma*, *Serrasalmus* and *Metynnis*, and the other characteristic of *Piaractus*. They are differentiated by the floccus of chromatin smallest and thinnest, by the midpiece and cytoplasmic sleeve shape and length of the last genus. Those of the genus *Piaractus* (Cruz-Landim *et al.*, 2003; current paper) are quite distinct, and have a great similarity with the spermatozoa of the genera *Salminus* and *Brycon* (Aires *et al.*, 2000; Romagosa *et al.*, 1999; Zaiden, 2000; Veríssimo-Silveira *et al.*, 2006). The type of chromatin condensation in floccus, the ovoid shape of the nucleus, the length of the midpiece, the mitochondria shape and distribution, and the presence of a well defined cytoplasmic sleeve response by this similarity. The main differences of the spermiatic cells among these three genera are the largest floccus of chromatin, the deepest nuclear fossa of *Salminus* and *Brycon*, and the shortest cytoplasmic sleeve, and the flagellar fins of *Piaractus*. *Piaractus* occupies a basal position in Serrasalminae along with *Mylossoma* and *Colossoma* (Ortí *et al.*, 1996, 2008; Calcagnotto *et al.*, 2005). The larger similarity of the spermatozoa of *Piaractus* to other basal characid taxa (Malabarba & Weitzman, 2003) represented by *Brycon* and *Salminus*, seems to represent plesiomorphic features shared with non-serrasalmines.

A particular characteristic observed in the flagellum of *Metynnis* differentiates it from the other serrasalmine genera: the presence of a tubule-vesicular compartment that runs alongside in part of length the flagella, instead of flagellar fins found in all other serrasalmine herein analyzed. This kind of vesicles, also observed in the initial portion of the flagella in *Serrasalmus maculatus* and the flagellar fins, may eventually be a different expression of the same character.

Between the two distinct patterns of spermiatic characteristics of the serrasalmine genera herein analyzed, the one common to *Mylossoma*, *Serrasalmus* and *Metynnis* seems to be derived. The second one, characteristic of *Piaractus*, is very similar to those found in other characids as *Salminus* and *Brycon*, and seems to constitute plesiomorphic features. These data permit to corroborate both morphological and molecular analyses that place *Piaractus* as a basal genus among serrasalmines. Considering that the relationship patterns in Characidae remain mostly unsolved, the information that can be offered by the spermatozoa characteristics in this group of fish became more and more significant and reinforced the potential of the kind of dates to the phylogenetic analyzes.

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