Arab Gulf Journal of Scientific Research, 24 (2), pp 58-74 (2006)

Review Paper Ref. No . 2357

Spermatogenesis and Spermatozoon Ultrastructure in the Nile Pebblyfish *Alestes dentex* (Teleostei: Characiformes: Alestidae)

مراحل التكوين والتركيب الدقيق للحيوان المنوى فى سمك الراى النيلى *Alestes dentex* (Teleostei: Characiformes: Alestidae)

عادل ع. ب. شاهين

Adel A. B. Shahin

Department of Zoology, Faculty of Science, Minia University, 61519 El-Minia, Egypt

To whom correspondence should be addressed: E-mail: abashahin@yahoo.com

Abstract: The ultrastructure of spermatogenesis and spermatozoon of *Alestes dentex* is described by using scanning and transmission electron microscopy. The testis is lobular in shape and spermatogenesis is of the unrestricted type. The germ cells are found in clusters within the seminiferous tubules and surrounded by a cytoplasmic processes of the Sertoli cells. Spermiogenesis is characterized by chromatin condensation, flagellum development, nuclear rotation, migration of the diplosome and mitochondria to the basal pole of the nucleus, nuclear indentation, nuclear fossa formation and loss of the excess cytoplasm. The mature spermatozoon is of the primitive type; type I aquasperm. The spermatozoon has no acrosome and has a rounded head with a heterogeneously electron-dense nucleus, which contains a deep axial nuclear fossa and a nuclear notch. The nuclear fossa contains the centriolar complex and part of the basal body of the axoneme. The short midpiece contains a mitochondrial ring, which consists of several unequal-sized and unevenly distributed mitochondria. The flagellum has the classical axoneme pattern of 9+2 and has no lateral fins or a membranous compartment. In addition, the spermatozoon has some peculiar features, which are not previously described in Characiformes and include the presence of two fibrous bodies anchoring the proximal centriole to the nucleus, a thick outer membrane of dense fibers separating the flagellum from the cytoplasmic canal and a basal foot and alar sheets attaching the basal body to the nucleus and plasma membrane. These findings suggest that the ultrastructural features of spermiogenesis and spermatozoa of *A. dentex* are synapomorphy of Ostariophysi, Perciformes, and Salmoniformes.

Key words: Spermatogenesis, sperm, *Alestes dentex,* ultrastructure, morphology, Characiformes.

المستخلص: قام هذا البحث بوصف التركيب الدقيق ومراحل التكوين للحيوان المنوى فى سمك الراى النيلى *Alestes dentex* بإستخدام الميكروسكوب الإليكترونى الماسح والنافذ. وقد وجد أن الخصية مفصصة الشكل، وأن مراحل تكوين الحيوان المنوى هى من النوع الغير محدد (أو اللانهائى) التكوين. وأن الخلايا المنبتة توجد فى مجموعات فى داخل الأنيبيبات المنوية، ومحاطة بالزوائد السيتوبلازمية لخلايا سيرتولى. وقد تميزت مراحل تميز الحيوان المنوى ببعض الخصائص مثل: تكثيف الكروماتين، تكون السوط أو الذيل، الدوران النووى، هجرة محورالجسم المركزى السوطى والميتوكوندريا إلى القطب القاعدى للنواة، الإنبعاج النووى، تكوين الحفرة النووية، والتخلص من السيتوبلازم الزائد. وقد وجد أيضا أن الحيوان المنوى هو من النوع البدائى (نوع ا، الحيوان المنوى المائى). ولايمتلك هذا الحيوان جسم قمى، وله رأس بها نواة مستديرة ذات كثافة إليكترونية غير متجانسة، وذات حفرة نووية محورية وعميقة تحوى الحبيبة أو الجسم المركزى المركب والجسم القاعدى للذيل، وبها أيضا ثلمة نووية. وتحوى القطعة الوسطى للحيوان المنوى على حلقة الميتوكوندريا التى تتكون من العديد من الميتوكوندريا المختلفة الحجم، والغير متجانسة التوزيع على الجانبين. ويمتلك الذيل التركيب الأساسى للقطعة المحورية (9+2)، ولايمتلك زعانف جانبية، أو أى تركيبات غشائية. وبالإضافة إلى ذلك فقد وجد أن الحيوان المنوى يمتلك بعض الخصائص الفريدة والتى لم توصف من قبل بين الكرسينيات، وهى تشمل: وجود جسمين ليفيين اللذين يربطان الحبيبة أو الجسم المركزى القريب بالنواة، وجود غشاء خارجى سميك من ألياف كثيفة يفصل القناة السيتوبلازمية عن الذيل، ووجود قدم قاعدى وشرائط إبطية تصل الجسم القاعدى والغشاء البلازمى بالنواة. وخلص البحث إلى خصائص التركيب الدقيق ومراحل تكوين الحيوان المنوى فى أسماك الراى مشتقة من تلك الموجودة فى الأوستاريوفيزى، البرشيات (الفرخيات)، والسلمونيات.

كلمات مدخلية: التركيب الدقيق، مراحل تكوين، الحيوان المنوى، *Alestes dentex* ، الكرسينيات .

Introduction

Spermatogenesis, in general, exhibits only limited fine structural variations in animal species; however, spermiogenesis shows a wide variety of patterns (Franzen 1970). In teleosts, two types (I and II) of spermiogenesis have been recognized (Mattei 1970). In type I, rotation of the nucleus occurs and the diplosome enters the nuclear fossa and the flagellum is symmetrically located, while in type II, there is no nuclear rotation, the diplosome remains outside the fossa and the flagellum is asymmetrically located. Depending upon the variation in these features, spermiogenesis has been used to provide worthwhile insights into the relationships of many fish groups, especially at or above the family level (Grier 1973, 1975, 1976, Jamieson and Grier 1993, Quagio-Grassiotto, *et al.* 2001a, b, 2003, Medina 2003). Accordingly, teleost spermatozoa are widely different in morphology (Jamieson 1991) and their structure is influenced by both the reproductive mode and the systematic position (Gardiner 1978, Grier, *et al.* 1978). Generally, they vary from aflagellate to biflagellate and have an enormous range of shapes, sizes, and structures; the number and location of organelles also vary (Baccetti, *et al.* 1984, Baccetti 1986, Jones and Butler 1988, Jamieson 1991, Mattei 1991, Shahin 2006 ab,c). Both light and electron microscopy of a wide spectrum of teleost spermatozoa have shown that although quite a number of similarities are found among congeneric species (Todd 1976, Brusle 1981, Baccetti, *et al.* 1984, Vari 1989, Matos, *et al.* 1998, Quagio-Grassiotto, *et al.* 2003), important morphological differences can also be found between different species (Ginzburg 1968, Mattei 1970, Gwo, *et al.* 1996, Burns, *et al.* 1998). These differences can, therefore be used to infer both the taxonomic and phylogenetic relationships among taxa (see, for example, Billard 1970, Mattei and Mattei 1974, Jamieson 1991, Baccetti, *et al.* 1984, Baccetti 1985, Lahnsteiner and Patzner 1990, Mattei 1991, Gwo and Arnold, 1992, Gwo, *et al.* 1992, 1993, 1994b, 1995, 1996, Gwo and Gwo 1993, Gwo 1995, Burns, *et al.* 1998, Medina 2003).

Despite the immense studies on fish spermatozoa, the majority of teleost spermatozoa remain unexamined. Moreover, there appears to be no published data describing the fine structure of the spermatozoa of the Nile characid fish. The goals of this study are to examine the ultrastructure of spermatogenesis and spermatozoon of the pebbly-fish *Alestes dentex,* which belong to one of the endemic families of Characoidei in Africa, the family Alestidae and to compare the present data on *A. dentex* with that available on other fish groups in general and Characiformes in particular.

Materials and Methods

In June, during the breeding season (April-September), mature males of *A. dentex* measuring 15-22 cm in total length, were collected from the Nile at El-Minia (Middle Egypt). Samples of testis and semen were fixed in 3% glutaraldehyde made in 0.12 M phosphate buffer (pH 7.4) for 1 hr at 4°C and postfixed in 1% osmium tetroxide for 1 hr. After fixation, samples were dehydrated in a graded ethyl alcohol series. Dehydrated samples of testis were cleared in propylene oxide and embedded in low viscosity Epoxy resin. Ultrathin sections were subsequently made on a LKB ultramicrotome with a glass knife and stained with drops of 2% uranyl acetate followed by lead citrate for 30 min. Sections were examined and photographed in JEOL JEM-100CX II TEM operated at 80 kV accelerating voltage. However, dehydrated samples of semen were critical point dried, coated with gold, and observed with JEOL JSM-5400LV SEM operated at 15 kV. Germ cells and spermatogenic stages were identified according to the criteria given by Gwo and Gwo (1993) and sizes of germ cells. Organelles and other structures were attained on the basis of 10 to 15 measurements from each category and mean and standard deviation values are given.

Results

The testis of *A. dentex* is lobular in shape and the germ cells are arranged in cysts or clusters within the seminiferous tubules. Spermatogenesis occurs in several places along the length of each tubule and the testis structure could be described as "unrestricted spermatogonial testicular type." Spermatogonia are found near the periphery along the length of the tubule, while spermatocytes, spermatids, and spermatozoa are found toward the interior. Sertoli cells are found surrounding the germ cells. Within the lobules, spermatogonia undergo numerous mitotic divisions producing cysts containing several spermatogonial cells. Depending upon the morphology and size of the nucleus, presence of organelles, and centriolar morphology, particularly the pericentriolar structures, germ cells are classified into the following types:

1. Spermatogonia

Primary spermatogonia are the smallest germ

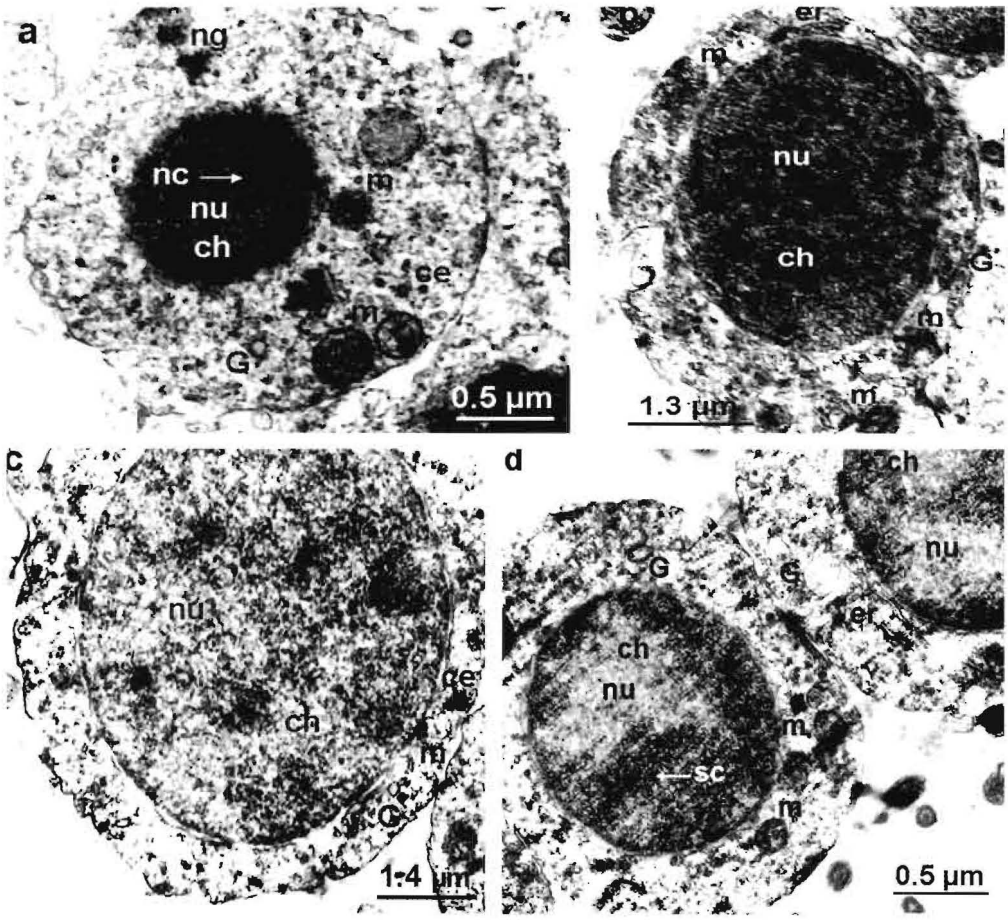

Fig. 1. Transmission electron micrographs in the spermatogenic cells of *A. dentex*. **(a)** Primary spermatogonium with a prominent nucleus (nu), well-defined nucleolus (nc) and electron-dense chromatin (ch). Mitochondria (m) are unevenly distributed in the cytoplasm and granular materials including nuage (ng) are distributed either free in the cytoplasm or associated with mitochondria and referred as cement (ce). **(b)** Secondary spermatogonium having a large nucleus and electron-dense granular chromatin. Note the cytoplasm contains several spherical mitochondria, Golgi vesicles (G) and endoplasmic reticulum (er). **(c)** Primary spermatocyte with a large nucleus that displays a clumped or slightly mottled chromatin. The cytoplasm contains mitochondria, cement and Golgi vesicles. **(d)** Secondary spermatocyte, which shows a small nucleus with granular and electron-dense mottled chromatin and synaptonemal complexes (sc). Mitochondria, Golgi vesicles and endoplasmic reticulum are clearly visible in the cytoplasm.

cells (Fig. 1a). They measure 1.6 ± 0.3 µm in diameter and possess a prominent dark rounded nucleus and an extensive cytoplasm. The cytoplasmic processes of the Sertoli cell surround either one or two spermatogonia. The nucleus is small (1.0 ± 0.1 µm in diameter), nearly central in position and contains darkly stained granular chromatin that exhibits irregular condensed patches. The nucleus contains one distinct nucleolus with fibrilar and granular chromatin. The cytoplasm contains granular materials that are either free (nugae) or associated with mitochondria (cement). Mitochondria are unevenly distributed throughout the cytoplasm, while the endoplasmic reticulum is concentrically organized in the peripheral cytoplasm.

Secondary spermatogonia produced by mitotic division of the primary spermatogonia are comparatively larger cells, 3.7 ± 0.4 µm in diameter, with less well-defined nucleoli, electron-dense material (nugae) and cement (Fig. 1b). The nucleus is 2.6 ± 0.7 µm in diameter and contains irregular patches of granular chromatin. Within the cysts, spermatogonial cells remain connected together by cytoplasmic bridges during mitotic division.

2. Spermatocytes

Primary spermatocytes are the largest germ cells (Fig. 1c). They measure 6.3 ± 0.01 µm in diameter and are distinguished by having a very large nucleus (5.7 ± 0.8 µm in diameter) and a dense granular chromatin. The nucleus appears clumped or slightly mottled in shape and without nucleolus. Secondary spermatocytes produced by the first meiotic division of the primary spermatocytes are comparatively smaller cells (Fig. 1d). They have about half of the size of the primary cell, 3.7 ± 0.3

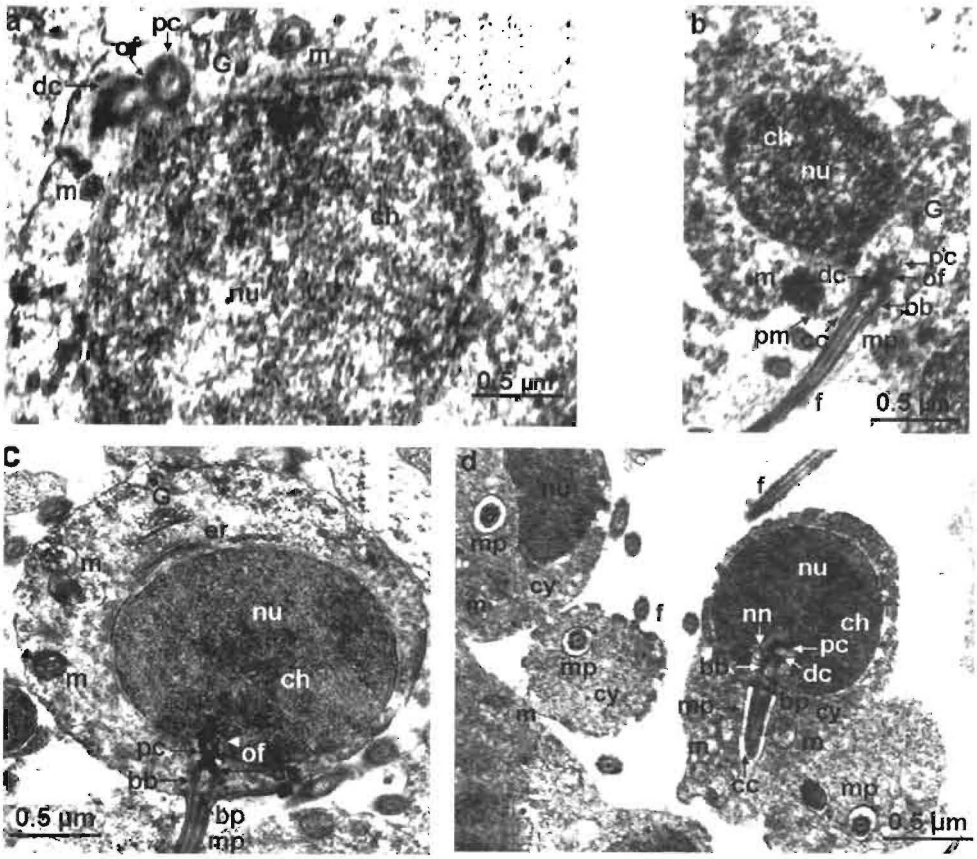

Fig. 2. Transmission electron micrographs through the early stages of spermiogenesis in *A. dentex*. **(a)** Transverse section showing the proximal (pc) and distal centrioles (dc) located at right angle to each other lateral to the nucleus (nu) and close to the plasma membrane and interconnected by osmiophilic filaments (of). Chromatin (ch) is condensed and mitochondria (m) and Golgi vesicles (G) appear close to the centriolar complex. **(b)** Longitudinal section showing the two centrioles interconnected by osmiophilic filaments and lie close to the plasma membrane (pm) and the basal body (bb) starts to form the flagellum (f), which extends parallel to the nucleus. A cytoplasmic canal (cc) is formed between the plasma membrane and the flagellum and extends longitudinally around the midpiece (mp). The mitochondria appear around the base of the flagellum and Golgi apparatus appears as vesicular cisternae around the centrioles. The nucleus is electron-dense with prominent patches of fine and coarse granular chromatin (ch). **(c)** Longitudinal section showing the nucleus with finely granular homogeneous chromatin. The nucleus becomes indented and a nuclear fossa (nf) (**arrowhead**) is formed. Note the diplosome-flagellar axis becomes perpendicular to the base of the nucleus due to the rotation of the nucleus. The proximal centriole is located within the nuclear fossa and the distal centriole gives rise to the basal body, which forms the flagellum. The basal body is traversed by the basal plate (bp) at its connection with the midpiece. Mitochondria, Golgi flattened cisternae and endoplasmic reticulum (er) are distributed within the residual cytoplasm. **(d)** Oblique transverse section (left) showing that the nucleus becomes more compact with increasing dense and thick chromatin and the 9 + 2 microtubular pattern of the axoneme in the midpiece region that is surrounded by the cytoplasmic canal. Longitudinal section (right) showing the rounded nucleus with increasing dense and thick chromatin filaments and the nuclear fossa is further expanded into the nucleus as far as one-fourth of the nucleus length and contains the centriolar complex and part of the basal body, the base of which is traversed by the basal plate. Note in the midpiece, the cytoplasmic canal separates the mitochondria from the flagellum. Most of the residual cytoplasm (cy) surrounds the midpiece.

µm in diameter, while the nucleus is relatively smaller, 2.6 ± 0.02 µm in diameter. Pachytene stage spermatocytes have clumped nuclear chromatin and synaptonemal complexes. The cytoplasm forms a narrow strand with irregular-shaped and an electron dense-matrix mitochondria.

3. Spermiogenesis

The polarization of germ cells starts at this stage. The nucleus moves to an eccentric position, while the cell organelles migrate and concentrate at the opposite pole of the cell. Early spermatids produced by second meiotic division of secondary spermatocytes have a small round nucleus (2.1 ± 0.9 µm in diameter) with granular chromatin distributed in small electron-dense patches of heterogeneous density, reduced cytoplasm and inconspicuous ribosomes (Fig. 2a). The spherical spermatids remain interconnected by cytoplasmic bridges,

which result from the incomplete cytokinesis of the mitotic and meiotic divisions. The centriolar complex appears lateral to the nucleus and close to the plasma membrane to form the flagellum (Figs. 2 a,b). Some mitochondria of spherical or ovoid shape are located near the centrioles. During the subsequent stage, the centrioles migrate to the basal pole of the nucleus (Fig. 2c). The process of spermiogenesis could be divided into the following stages based on the formation of the flagellum, rotation and condensation of the nucleus and loss of the superfluous cytoplasm (exocytosis).

4. Formation of the flagellum

In this stage, the two centrioles, each with nine triplet microtubules, are arranged at right angles to each other and appear to be interconnected by osmiophilic filaments. Both centrioles lie close to the plasma membrane and the basal body of the distal centriole starts to form the flagellum (Fig. 2b). The flagellum reveals a typical axonemal configuration with two single central and nine double peripheral microtubules. The axoneme then detaches itself from the perinuclear region and extends backward parallel to the nucleus, bringing with it the plasma membrane and thereby leaving a space, the cytoplasmic canal, between the plasma membrane and the flagellum (Fig. 2b). The diplosome-flagellar axis is tangential to the nucleus and the mitochondria aggregate around the base of the flagellum. Golgi apparatus appears as vesicular cisternae. The nucleus is electron-dense with prominent patches of fine and coarse granular heterochromatin. The cell outline appears very irregular in shape and the cytoplasmic bridges become narrower than in the previous stages.

5. Rotation and condensation of the nucleus

The cells in this stage display a finely granular appearance because of the homogeneity of chromatin (Figs. 2c,d). The nucleus becomes indented and a nuclear fossa is formed. Due to the rotation of the nucleus (90°) the diplosome-flagellar axis becomes perpendicular to the base of the nucleus. The proximal centriole is then located within the nuclear fossa (Fig. 2c). A nuclear notch appears in the region between the proximal and distal centrioles (Fig. 2d). In addition, two fibrous bodies, each of which consists of osmiophilic disks alternating with lighter material, appear perpendicular to each other above the proximal centriole within the nuclear fossa (Figs. 3a,b,c,d). The two bodies are interconnected with osmiophilic filaments and the upper body attaches to the nucleus with two bands of osmiophilic filaments (Fig. 3c); similarly, the lower body connects with the proximal centriole (Fig. 3d). Moreover, a basal foot appears laterally in the basal part of the distal centriole and anchors itself to the nucleus (Figs. 4a,b,c). The axoneme appears surrounded by mitochondria, which are separated from the flagellum by the cytoplasmic canal (Fig. 4a). As development proceeds, the size of the spermatids decreases and the intercellular spaces enlarge to form a lumen in the cyst.

6. Exocytosis

The nucleus becomes more compact with increasing dense and thick chromatin filaments (Fig. 4b). The nuclear fossa is further expanded into the nucleus, as far as one-fourth of the nucleus length, and completely surrounds the centriolar complex (Figs. 4a,b,c). As the nuclear fossa develops, the nucleus condenses and a small nuclear notch appears at the level of the proximal centriole, which gradually increases and becomes filled with electron-dense material (Figs. 4a,b,c). The cell begins to discard the excess cytoplasm, leaving the so-called residual bodies of various sizes and shapes in the intercellular spaces (Fig. 4d).

7. Spermatozoon

The mature spermatozoon is a relatively simple elongated cell composed of a head, a short midpiece, and a relatively long tail or flagellum (Fig. 5a). The head is spherical in shape, 1.9 ± 1.0 μm in diameter, and has no acrosome. The nucleus is spherical, 1.7 ± 0.7 μm in diameter, and covered by a typical double-layered undulating nuclear envelope. The nuclear envelope and the plasma membrane are applied tightly to the anterior part of the nucleus (Fig. 5b). However, the posterior part is indented by a nuclear fossa, the length of which is about more than one half of the nucleus diameter (Figs. 5b,c,d). The fossa is bell-shaped in longitudinal sections, circular in transverse sections and contains the centriolar complex, part of basal body and cytoplasm. A peculiar nuclear notch with some electron-dense material connects the lower fibrous body to the nucleus (Fig. 5d). The chromatin is heterogeneously granular, highly electron-dense and contains tightly packed fibers and irregular small clear lacunae (Figs. 5b,c,d). The proximal and distal centrioles occupy only the distal part of the nuclear fossa are perpendicular to each other and lie at right angle to the base of the head (Fig. 5d). Both

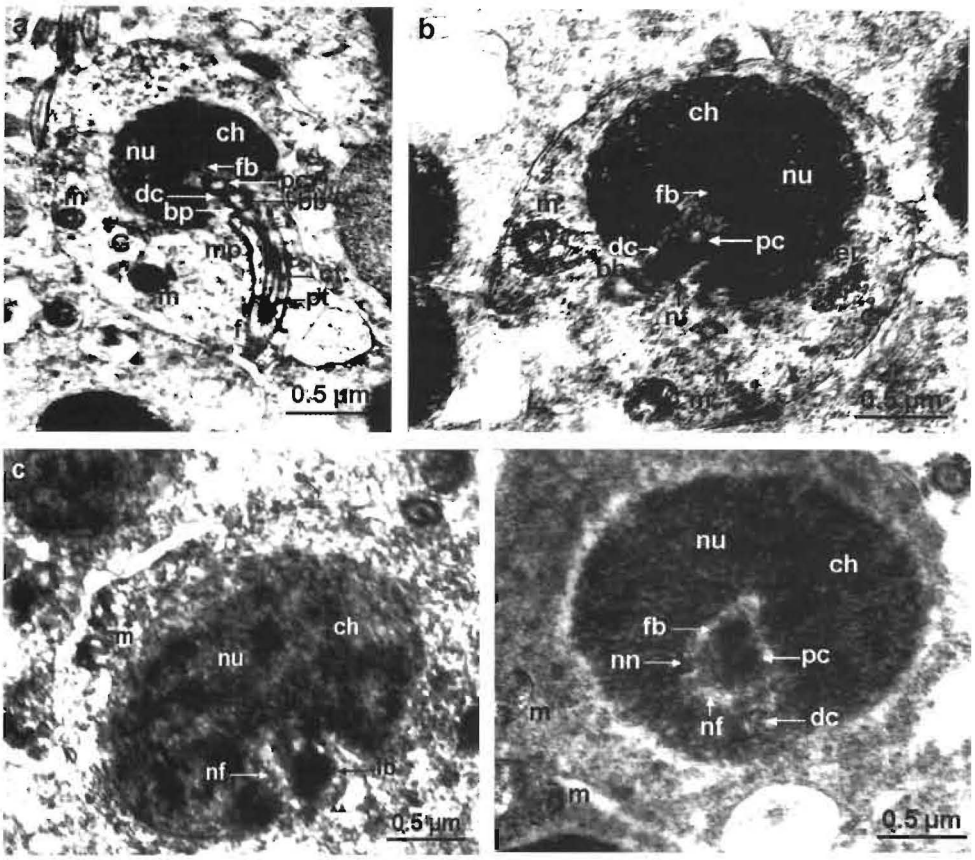

Fig. 3. Transmission electron micrographs in the late spermatids of *A. dentex*. **(a)** Longitudinal section showing that the nucleus (nu) is more compact and chromatin (ch) is more condensed with dense and thick filaments. The fibrous body (fb) lies above the proximal centriole (pc) and connects with the nucleus. The nuclear fossa (nf) completely surrounds the centriolar complex and part of the basal body (bb). The flagellum (f) consists of nine peripheral (pt) and two central (ct) microtubular filaments. The transitional region (**small arrow**) between the basal body (bb) and the midpiece (mp) lacks the central microtubules. **(b)** Oblique longitudinal section at the level of the head showing the nucleus with heterogeneous chromatin, the fibrous body lies above the proximal centriole and the nuclear fossa contains the proximal and the distal (dc) centrioles and part of the basal body. **(c)** Oblique transverse section through the head region showing the nucleus with heterogeneous chromatin and the upper fibrous body anchors to the nucleus by two bands of electron-dense filaments (**arrowheads**). Double **arrowheads** refer to the location of the lower fibrous body. **(d)** Transverse section through the head region at the level of the lower fibrous body showing that the latter lies just above the proximal centriole and interconnected with osmiophilic filaments and the nuclear fossa is circular. bp = basal plate, er = endoplasmic reticulum, G = Golgi apparatus, m = mitochondria, nn = nuclear notch.

centrioles display a characteristic nine-microtubular triplet pattern. An osmiophilic ring embedded in an electron-dense material surrounds the anterior end of the distal centriole. The triplets of the proximal and distal centrioles are interconnected by means of osmiophilic filaments (Figs. 6b,c). Two fibrous bodies, upper and lower, consisting of osmiophilic disks alternating with lighter material lie perpendicularly above the proximal centriole (Fig. 6a). These two dense bodies give rise to short electron-dense fibers, which connect them together with the proximal centriole and anchor the proximal centriole to the nucleus (Fig. 5d). However, the distal centriole, which forms the basal body of the axoneme, extends from the level of the anterior end of the cytoplasmic canal to the basal nuclear fossa and its base is traversed by a prominent basal plate (Figs. 5b,c,d). A striated basal foot attaches laterally in the midregion of the basal body and anchors it to the nucleus (Figs. 5b,c). Alar sheets radiate from the basal body triplets and attach it to the plasma membrane (Fig. 5c).

The midpiece is short, 0.5 µm in length and 0.9 µm in diameter, and contains a mitochondrial ring (Figs. 5b,c). The latter consists of several (approximately four to five) unequal-sized spherical or ovoid mitochondria, which measure about 0.6 ± 0.01-0.8 ± 0.7 µm in diameter and are completely separated from the midpiece by the cytoplasmic canal (Figs. 6d, 7a). The distribution of mitochondria is irregular on both sides of the midpiece (Figs. 5b,c,7b). The mitochondria have a cristae and an electron-dense matrix and are separated from the cytoplasmic canal by two closely

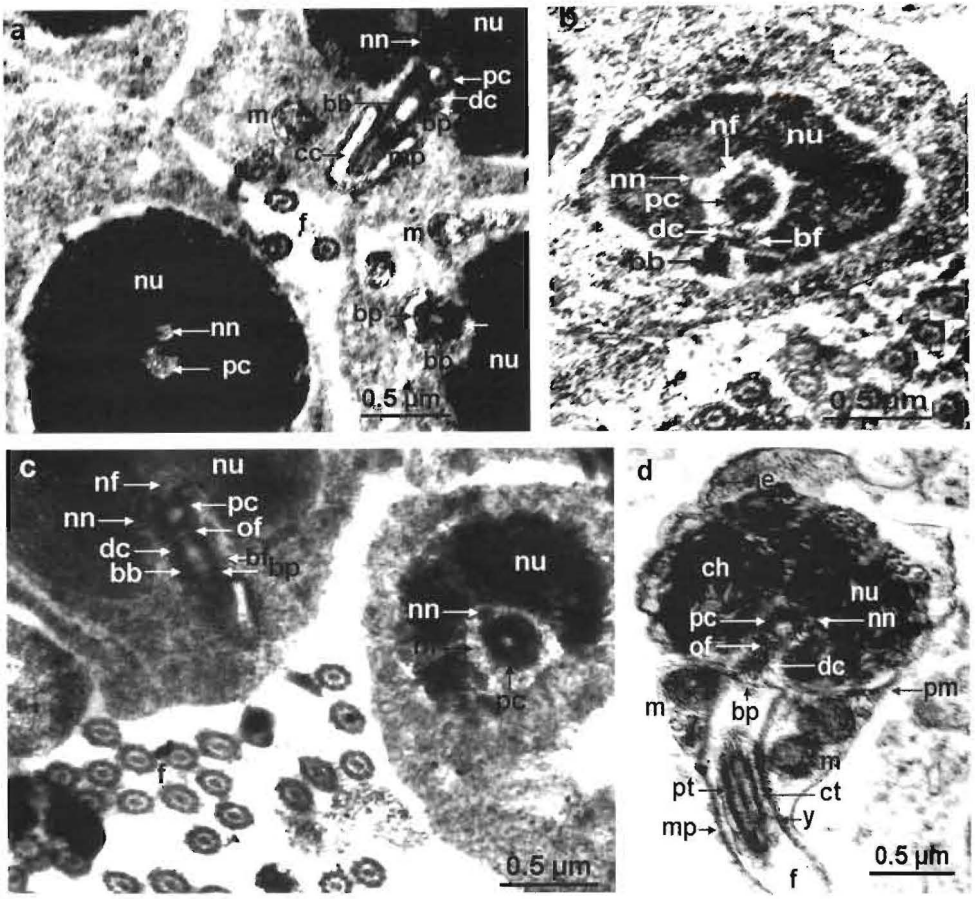

Fig. 4. Transmission electron micrographs in the late spermatids of *A. dentex*. **(a)** Oblique longitudinal section (upper) showing the nuclear notch (nn) that anchors the proximal centriole (pc) to the nucleus (nu) and the basal foot (bf) (**white arrow**) that extends on both sides from the lateral side of the basal body (bb). Transverse sections in the head region at the level of the extension of the nuclear notch from the proximal centriole (lower left) and the basal body (lower right) showing their microtubular structure pattern. **(b)** Transverse section in the head region at the level of the proximal centriole showing the nucleus with circular nuclear fossa (nf), the nuclear notch and the basal foot that extends on both sides from the basal body and anchors to the nucleus. Note the location of the distal centriole (dc). **(c)** Oblique longitudinal section (left) showing the nucleus with heterogeneous chromatin (ch), nuclear notch and nuclear fossa that contains the centriolar complex and the basal body with its basal foot. Oblique transverse section (right) at the level of the proximal centriole showing the nucleus with the circular nuclear fossa and nuclear notch. **(d)** Longitudinal section in the early spermatozoon showing the spherical nucleus with heterogeneously granular and electron-dense chromatin, which contains tightly packed fibers and irregular small clear lacunae and covered by a double-layered undulating nuclear envelope (ne) that applies tightly to the plasma membrane (pm). Note that the mitochondria (m) lie close to the basal pole of the nucleus and surround the midpiece (mp) and the peripheral microtubular (pt) doublets of the axoneme are connected to the plasma membrane by a Y-shaped electron-dense body (y). bp = basal plate, cc = cytoplasmic canal, ct = central microtubules, dc = distal centriole, f = flagellum, of = osmiophilic filaments.

apposed membranes (Figs. 5b,c, 6d, 7a). Another membrane of dense fibrous layer extends from the posterior end of the midpiece collar to the anterior end of the cytoplasmic canal in close vicinity to the flagellar plasma membrane and it separates the midpiece from the cytoplasmic canal (Figs. 5b,c, 6d, 7a,b).

The flagellum measures 28.5 ± 1.02 µm in length and 0.3 ± 0.4 µm in diameter and is surrounded by the flagellar plasma membrane. In the transitional region between the base of the axoneme and the basal plate, the axoneme consists of nine double outer tubules and lacks the central tubules (Fig. 5c). Distal to these regions, the flagellum has the classic nine double outer and two single central microtubular constructions (Figs. 6d, 7a,b). The doublets are connected to the flagellar plasma membrane by short Y-shaped bridges. The central tubules are surrounded by a thin sheath and appear to be interconnected by a single strand. Each of the nine outer doublets consist of subfibers A and B. Two dynein arms arise from subtubule A of each doublet and extend toward the next tubule (Figs. 6d, 7a,b).

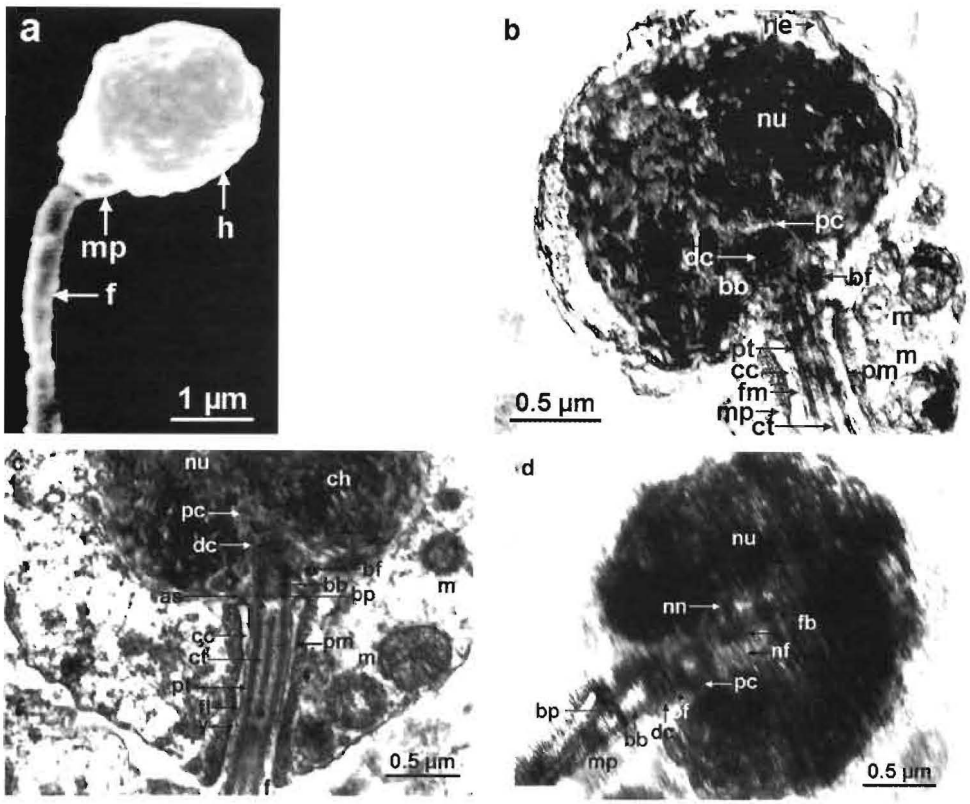

Fig. 5. Electron micrographs of the mature spermatozoon in *A. dentex*. **(a)** Scanning electron micrograph showing that the sperm has a spherical head (h), a short midpiece (mp) and a tail or flagellum (f), but there is no acrosome. (b-d) Transmission electron micrographs. **(b)** Longitudinal section showing the nucleus (nu) with heterogeneous electron-dense chromatin (ch) that contains tightly packed fibers and irregular small lacunae and covered by a double-layered undulating nuclear envelope (ne) that is and the plasma membrane (pm) are applied tightly to the anterior part of the nucleus. The posterior part of the nucleus is indented by a nuclear fossa (nf), which has a length of about more than one half of the nucleus diameter. The fossa contains the proximal (pc) and distal centrioles (dc) and part of basal body (bb). Note the basal foot (bf) extends from the basal body, the cytoplasmic canal (cc) surrounds the midpiece and separates it from the mitochondria (m) and the central (ct) and peripheral (pt) microtubules of the midpiece region. **(c)** Enlarged part of the posterior part of the nucleus and midpiece showing the basal foot and alar sheets (as) extending on both sides of the basal body and attach it to the nucleus and the plasma membrane. Note that mitochondria are unevenly distributed on both sides of the midpiece and two closely apposed membranes run along between the cytoplasmic canal and the outer layer of mitochondria ring, in addition to another membrane of dense fibrous layer (fl) that extends from the posterior end of the midpiece collar to the anterior end of the cytoplasmic canal in close vicinity to the flagellar plasma membrane (fm) and separates the midpiece from the cytoplasmic canal. **Arrowhead** refers to the transitional region that is free of central microtubules between the basal body and the midpiece. The peripheral microtubules are connected to the plasma membrane by a Y-shaped electron-dense body (y). **(d)** Longitudinal section showing clearly the two fibrous bodies (fb), upper and lower, interconnected with osmiophilic filaments (of) and anchor the proximal centriole to the nucleus. Note the nuclear notch (nn) which connects the lower fibrous body with the nucleus, the osmiophilic filaments interconnect the proximal centriole with distal centriole, the length of the nuclear fossa, which is about more than one half the length of the nucleus, and the basal part of the basal body is traversed by the basal plate (bp).

Discussion

Earlier stages of spermatogenesis in *A. dentex* do not show any special peculiarities; nevertheless, its spermiogenic characters are consistent with those reported for type I aquasperm (Mattei, 1970; Jamieson, 1991). Rotation of the nucleus is 90°, the diplosome enters the nuclear fossa and the flagellum is symmetrically located. A similar rotation of the nucleus has been found in many other teleost taxa (for review, Shahin, 2006 a). On the contrary, rotation of the nucleus either occurs only to a slight degree as in *Cyprinus carpio*, *Esox lucius* (Billard, 1986), *Oreochromus niloticus* (Lou and Takahashi, 1989) and *Paracheirodon innesi* (Jamieson, 1991) or does not occur entirely as in *Liza aurata* (Brusle, 1981), *Diapoma speculiferum* and *Diapoma* sp. (Burns, *et al.* 1998), *Mimagoniates barberi* (Pecio and Rafiński, 1999), *Acestrorhynchus falcatus* (Matos, *et al.* 2000), *Merluccius merluccius* (Medina, *et al.* 2003) and in *Malapterurus electricus*, *Chrysichthys auratus* and *Synodontis schall* (Shahin, 2006 a,b,c). In these cases, except in the three latter catfish (Shahin, 2006 a,b,c), the

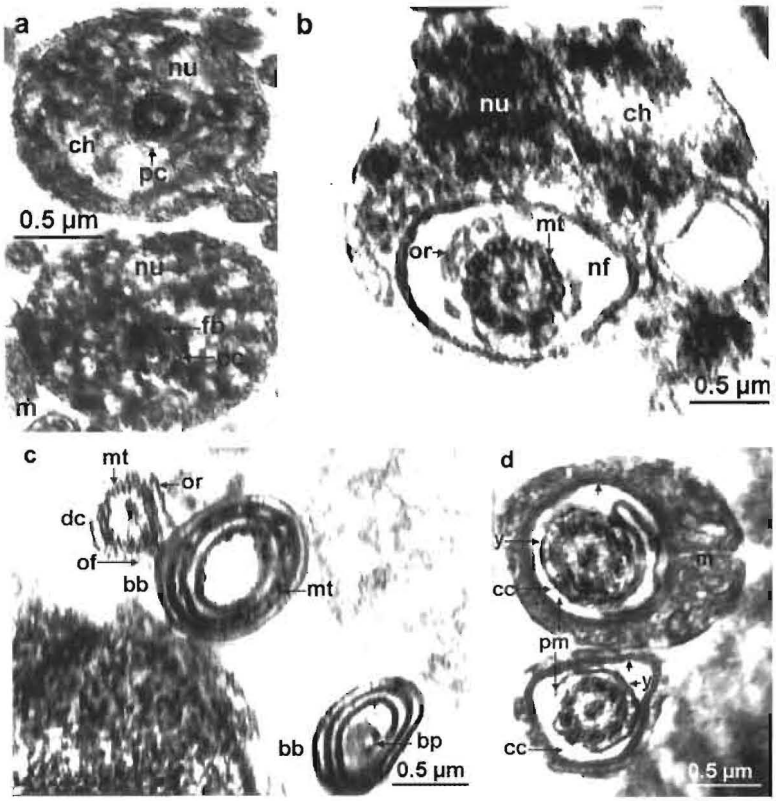

Fig. 6. Transmission electron micrographs in the head and midpiece of the mature spermatozoon of *A. dentex*. **(a)** Two transverse sections at the level of the proximal centriole (pc) (upper) showing the nucleus (nu) with tightly packed fibers and irregular small lacunae in the chromatin (ch) and at the level of the location of the lower fibrous body (fb) above the proximal centriole (lower). **(b)** Transverse section at the level of the basal region of the distal centriole (dc) showing the typical 9 + 2 construction pattern, which is surrounded by an osmiophilic ring (or) of electron-dense material that fills the nuclear fossa (nf). All the nine outermost microtubules of the triplets of the distal centriole disappear proceeding backwards. **(c)** Two transverse sections at the level of the distal centriole and the basal body (bb) (upper) and at the level of the basal plate (bp) of the basal body (lower) showing their microtubular construction pattern (mt). **(d)** Transverse section through the midpiece (mp) showing the cytoplasmic canal (cc) separating the mitochondria (m) from the midpiece. Note that the mitochondria are bounded by an outer and inner membrane (**arrow**) and exhibit transverse cristae and each of the peripheral microtubular doublets of the midpiece is connected to the plasma membrane by a Y-shaped link (y).

diplosome remains outside the nuclear fossa and the flagellum is asymmetrically located, i.e. it inserts laterally to the head (type II; Mattei, 1970). However, *M. electricus*, *C. auratus* and *S. schall*, Shahin (2006 a,b,c) describes that the nuclear rotation is absent, the diplosome enters the medial nuclear fossa and the flagellum is symmetrically located. Quagio-Grassiotto *et al.* (2003) mentioned that the position of the nuclear fossa and accordingly the attachment of the flagellum to the nucleus depend upon the rotation of the nucleus. Therefore, when the nuclear rotation is incomplete, the nuclear fossa is eccentric and so is the flagellum, which is perpendicular to the nucleus (Matos, *et al.* 1998; Jamieson, 1991; Burns, *et al.* 1998; Magalhães, 1998; Andrade, *et al.* 2001; Quagio-Grassiotto, *et al.* 2001 b, 2003). If rotation is complete (90°), as in *A. dentex* examined herein and in some other characids (Mattei, *et al.* 1995; Aires, 1998; Romagosa, *et al.* 1999; Zaiden, 2000; Veríssimo-Silveira, 2003), the nuclear fossa is medial and the flagellum is medial and perpendicular to the nucleus. However, when the nucleus does not rotate, the nuclear fossa is either lateral in position or may be also absent, thus the flagellum is parallel to the nucleus (Burns, *et al.* 1998; Matos, *et al.* 2000), or it is medial in position and the flagellum is perpendicular to the nucleus (Shahin, 2006 a,b,c). Moreover, Quagio-Grassiotto *et al.* (2003) pointed out that the position of the centriolar complex is related to the shape of nuclear fossa. When the nuclear fossa is deep, the centriolar complex is located inside it. If the nuclear fossa is of the moderate type, it may contain part or the entire centriolar complex or only one of the centrioles, while the other one lies outside. But if it is completely absent, the centriolar complex usually lies close to the nucleus.

According to Quagio-Grassiotto *et al.* (2003), the nuclear fossa in *A. dentex* is of the deep type,

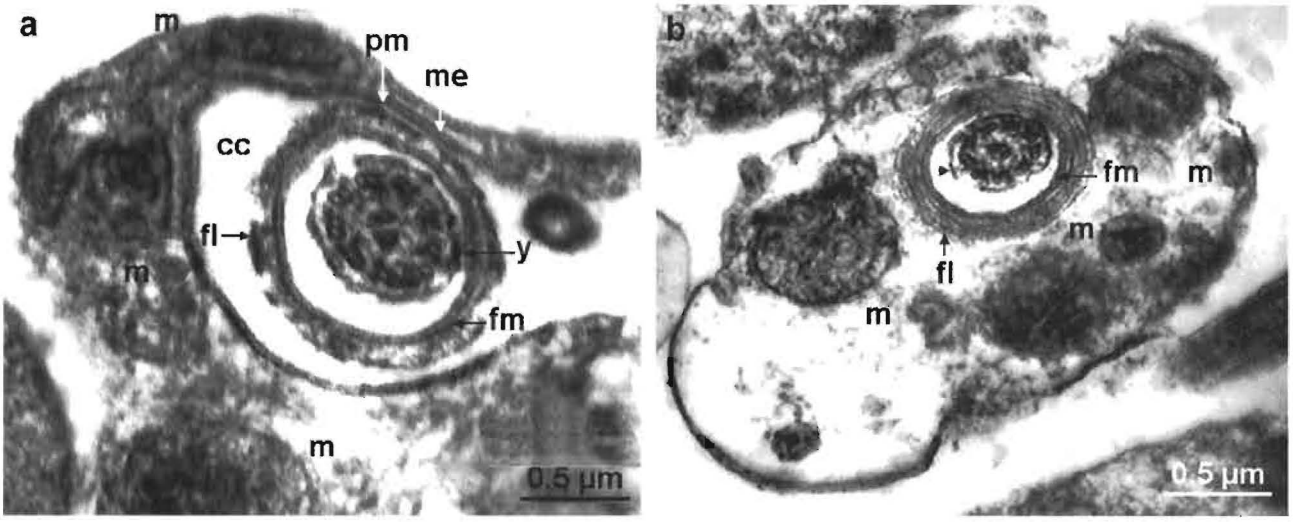

Fig. 7. Transmission electron micrographs in the midpiece of the mature spermatozoon of *A. dentex*. **(a)** Transverse section showing the 9 + 2 microtubular pattern of the axoneme in this region, each of the peripheral microtubular doublets (pt) is connected to the plasma membrane (pm) by a Y-shaped link (y). Note the double-layered flagellar membrane (fm) and its outer fibrous layer (fl) surrounding the plasma membrane and the mitochondrial envelope (me) that lies in close vicinity to the plasma membrane and separates the mitochondria (m) from the cytoplasmic canal (cc). **(b)** Transverse section showing several mitochondria (rarely 6) around the midpiece (mp) region. Note the double-layered flagellar membrane and its outer the fibrous layer, which surround the plasma membrane. **Arrowhead** refers to the Y-shaped link that connects each of the peripheral microtubular doublets with the plasma membrane.

since it has more than one half of the nuclear diameter in length and contains the centriolar complex and part of the basal body of the axoneme. The presence of a shallow to deep nuclear fossa, containing the centriolar complex and the proximal portion of the flagellum, is also common in the spermatozoa of many viviparous and some oviparous taxa (for more details, see Gwo, *et al.* 1993; Quagio-Grassiotto, *et al.* 2003; Shahin, 2006 a,b,c). Nevertheless, the nuclear fossa is moderately developed in the curimatid species (Matos, *et al.* 1998; Quagio-Grassiotto, *et al.* 2003), *Hoplias malabaricus* (Quagio-Grassiotto, *et al.* 2001 b) and in the subfamilies Aphyocharacinae (Burns, *et al.* 1998) and Myleinae (Matos, *et al.* 1993), while it is poorly developed in *Epinephelus malabaricus* and *Plectropomus leopardus* (Gwo, *et al.* 1994 b). It is completely absent in most oviparous and some viviparous species (Billard, 1970; Fribourgh, *et al.* 1970; Mattei and Mattei, 1974; Todd, 1976; Brusle, 1981; Gwo, 1989; Gwo and Arnold, 1992; Thiaw, *et al.* 1990; Guan and Afzelius, 1991; Burns, *et al.* 1998; Pecio and Rafiński, 1999; Matos, *et al.* 2000; Medina, *et al.* 2003).

On the other hand, the arrangement of the centriolar complex is variable in many cypriniform species; therefore, it is considered a species-specific feature (Mansour, *et al.* 2002). The proximal centriole may be anterior or lateral to the distal centriole. In either case, it may be coaxial, parallel, oblique or perpendicular to the distal centriole. In *A. dentex* spermatozoa, the two centrioles are arranged perpendicularly to each other and the proximal one lies anterior to the distal centriole. Similar conditions have been described in *Acanthopagrus schlegeli* (Gwo and Gwo, 1993; Gwo, *et al.* 1993), *A. latus* (Gwo, 1995), *Chanos chanos* (Gwo, *et al.* 1995), *Diplomystes mesembrinus* (Quagio-Grassiotto, *et al.* 2001 a), *M. merluccius* (Medina, *et al.* 2003) and in *M. electricus*, *C. auratus* and *S. schall* (Shahin, 2006 a,b,c). The two centrioles are oriented at right angles to each other in *Anguilla japonica* (Gwo, *et al.* 1992), *Plecoglossus altivelis* (Gwo, *et al.* 1994 a), *E. malabaricus* and *P. leopardus* (Gwo, *et al.* 1994 b) and *Oncorhynchus masou formosanus* (Gwo, *et al.* 1996). Among Characiformes variable orientation of the centrioles has been reported. For example, the proximal centriole is antero-lateral and slightly oblique to the basal body (Jamieson, 1991), lateral, oblique and distant from the distal centriole (Mattei, *et al.* 1995) or anterior, coaxial and slightly oblique to the basal body (Quagio-Grassiotto, *et al.* 2001 b). Moreover, it is anterior, lateral and perpendicular to the basal body (Matos, *et al.* 2000), anterior, medial and perpendicular to the basal body (Aires, 1998; Burns, *et al.* 1998; Pecio and Rafiński, 1999; Romagosa, *et al.* 1999; Zaiden, 2000), or anterior, medial to slightly lateral, and at a right or slightly oblique angle relative to the distal centriole (Quagio-Grassiotto, *et al.* 2003).

Furthermore, the midpiece exhibits two

situations among teleosts, one of which is at the posterior end of the nucleus as in *A. dentex* examined herein, many other teleosts (Jamieson, 1991; Mattei, 1991; Shahin, 2006 a,b,c) and some Characiformes (Jamieson, 1991; Matos, *et al.* 1993, 1998; Mattei, *et al.* 1995; Burns, *et al.* 1998; Andrade, *et al.* 2001; Quagio-Grassiotto, *et al.* 2001 b, 2003). In the other situation, the midpiece is located laterally to the nucleus as in some members of Characiformes (Burns, *et al.* 1998; Pecio and Rafiński, 1999; Matos, *et al.* 2000).

In *A. dentex*, the midpiece is short and contains a short cytoplasmic canals enveloped by the cytoplasmic sheaths that is formed as a thin cytoplasmic projection (Jamieson, 19991; Mattei, 1991). Similar cases have been recorded in the majority of Characiformes (Magalhäes, 1998; Matos, *et al.* 1998, 2000; Andrade, *et al.* 2001; Quagio-Grassiotto, *et al.* 2003) as well as in many other teleosts (for review, see Gwo, *et al.* 1993; Quagio-Grassiotto, *et al.* 2003; Shahin 2006 a,c). However, long midpiece and long cytoplasmic canal have been observed in some characids (Jamieson, 1991; Matos, *et al.* 1993; Aires, 1998; Romagosa, *et al.* 1999; Zaiden,-2000; Veríssimo-Silveira, 2003). Moreover, a long midpiece and long cytoplasmic sheath attached to one side of the nucleus have been found in the species of Glandulocaudinae (Burns, *et al.* 1998) except species of *Mimagoniates* (Burns, *et al.* 1998; Pecio and Rafiński, 1999) that have a short cytoplasmic sheath. However, a long midpiece with no cytoplasmic sheath has been found in the inseminating *Macropsobrycon uruguayanae* (Burns, *et al.* 1998).

In the spermatozoa of *A. dentex*, two closely apposed membranes run in the vicinity of the plasma membrane between the outer mitochondrial envelope and the cytoplasmic canal membrane. A similar structure has been described in *Thymallus thymallus* (Lahnsteiner, *et al.* 1991), *A. schlegeli* (Gwo and Gwo, 1993; Gwo, *et al.* 1993), *A. latus* (Gwo, 1995) and *M. merluccius* (Medina, *et al.* 2003). One membrane has been found in some other species (Brusle, 1981; Billard, 1983; Gwo, 1989; Gwo and Arnold, 1992; Gwo, *et al.* 1994 b; Gwo, *et al.* 1995; Gwo, *et al.* 1996; Burns, *et al.* 1998; Pecio and Rafiński, 1999; Burns, *et al.* 1998; Quagio-Grassiotto, *et al.* 2001 a; Quagio-Grassiotto, *et al.* 2003). Conversely, the cytoplasmic canal is absent in *P. altivelis* (Gwo, *et al.* 1994 a), *Citharinus* sp. (Mattei, *et al.* 1995), *H. malabaricus* (Quagio-Grassiotto, *et al.* 2001 b) and *C. auratus* (Shahin, 2006b). Mattei (1970) mentioned that the cytoplasmic canal is formed during spermiogenesis by the movement of the centriolar complex towards the nucleus, taking with it the plasma membrane and the initial segment of the flagellum.

The situation of mitochondria shows considerable variation among teleosts. In the spermatozoa of most taxa (Shahin, 2006 a,c) including *A. dentex* examined herein, mitochondria are located adjacent to the caudal pole of the nucleus and surround the initial segment of the axoneme and are separated from it by the cytoplasmic canal. Similar location of mitochondria has also been reported among subfamilies of Characidae, particularly those that have a long midpiece, such as Myleinae (Matos, *et al.* 1993), Bryconinae (Aires, 1998; Romagosa, *et al.* 1999, Zaiden, 2000), *Hyphessobrycon innesi* of the Tetragonopterinae (Jamieson, 1991) and Salmininae (Veríssimo-Silveira, 2003), where they are grouped in the anterior third of the midpiece around the initial region of the axoneme and separated from it by the cytoplasmic canal. The same arrangement has been observed also in an inseminating species of the Cheirodontinae (Burns, *et al.* 1998). In the species of the Glandulocaudinae, the elongate mitochondria are grouped and located close to the posterior end of the nucleus (Burns, *et al.* 1998; Pecio and Rafiński, 1999). The Acestrorhynchidae have few elongate mitochondria located around the nucleus and around the initial region of the axoneme and are separated from the axoneme by the cytoplasmic canal. However, mitochondria are found in the nuclear indentation in the flounder (Jones and Butler ,1988), many blenniid species (Lahnsteiner and Patzner, 1990; Silveira, *et al.* 1990), and several eels (Mattei and Mattei, 1974; Todd, 1976; Gwo, *et al.* 1992). In the citharinid species, mitochondria are located close to the nucleus near the centriolar complex (Mattei, *et al.* 1995). In *P. altivelis* (Gwo, *et al.* 1994 a) and *H. malabaricus* (Quagio-Grassiotto, *et al.* 2001 b), due to the absence of the cytoplasmic canal, a single mitochondrion in the former species or several mitochondria in the latter species, are situated close to the nucleus and laterally in relation to the flagellum. Moreover, in *C. auratus* mitochondria are located at the basal pole of the nucleus and separated from the axoneme by the inner mitochondrial envelope due to the absence of the cytoplasmic canal (Shahin, 2006 b).

In addition, the number and distribution of mitochondria are frequently variable among teleosts; they range from a single mitochondrion to several (up to ten) mitochondria. For example, one mitochondrion has been found lying either in a concavity in the anterior end of the nucleus as in *A. japonica* (Gwo, *et al.* 1992), lateral to the flagellum as in *P. altivelis* (Gwo, *et al.* 1994 a) or encircling

the base of the flagellum as in *C. chanos* (Gwo, *et al.* 1995), *O. m. formosanus* (Gwo, *et al.* 1996), *D. mesembrinus* (Quagio-Grassiotto, *et al.* 2001 a) and *C. auratus* (Shahin, 2006 b). It has been reported that there are three mitochondria in *A. latus* (Gwo, 1995), four in *A. schlegeli* (Gwo and Gwo. 1993; Gwo, *et al.* 1993), five (rarely six) in *E. malabaricus* and *P. leopardus* (Gwo, *et al.* 1994b) and *C. auratus* and *S. schall* (Shahin, 2006 b,c) and several, up to ten mitochondria, in *M. merluccius* (Medina, *et al.* 2003) or fifteen in *M. electricus* (Shahin, 2006 a), which surround the base of the flagellum. Among Characiformes, however, Burns, *et al.* (1998), Pecio and Rafiński (1999), and Quagio-Grassiotto, *et al.* (2001 b, 2003) reported that there is either one mitochondrion that initially extends laterally to the flagellum to one side of the nucleus or several mitochondria, as the case in *A. dentex* examined herein, which surround the base of the flagellum at the posterior part of the nucleus.

Development of an acrosomal complex (the acrosomal vesicle and the subacrosomal material) from the functions of smaller Golgi-derived proacrosomal vesicles is not apparent in *A. dentex*. The Golgi apparatus appears at the posterior region of the diplosome during spermiogenesis and disappears at the end of spermiogenesis. Absence of the acrosome is very common in the majority of teleosts. An acrosomal vesicle has been observed in *O. mykiss* (Billard 1983).

In *A. dentex*, progressive condensation of the chromatin occurs during spermiogenesis. In early stages, the chromatin appears as irregular aggregations of coarse and dense granules scattered throughout the nucleus. This is followed with gradual coalescence of the thick chromatin strands into denser aggregations. Changes in the nuclear chromatin condensation pattern during spermiogenesis have previously been reported in many other teleosts (see Gwo and Gwo, 1993; Shahin, 2006 a,b,c). Iatrou and Dixon (1978) interpreted the progressive alterations of chromatin packing as the result of the transformation of nuclear basic proteins.

Moreover, spermatozoan head nuclear chromatin is widely different in appearance among fish and it varies from dense, homogeneous and compact to granular and heterogeneous (for review, see Gwo and Gwo, 1993; Shahin, 2006 a,b,c). In *A. dentex*, the spermatozoon chromatin appears heterogeneous, highly electrondense and contains tightly packed fibers and irregular small clear lacunae. Similar appearance of chromatin has been found in some Characiformes (see Matos, *et al.* 1993; Mattei, *et al.* 1995; Aires, 1998; Burns, *et al.* 1998; Romagosa, *et al.* 1999; Zaiden, 2000; Veríssimo-Silveira, 2003). In contrast, among other members of the Characiformes, spermatozoa exhibit thick fibers of homogeneously condensed chromatin (Jamieson, 1991; Mattei, *et al.* 1995; Burns, *et al.* 1998; Magalhães, 1998; Matos, *et al.* 1998, 2000; Pecio and Rafiński, 1999; Quagio-Grassiotto, *et al.* 2001 b, 2003).

In *A. dentex*, the nucleus is spherical. A similar appearance of the nucleus has been recorded in some characids (Jamieson, 1991; Mattei. 1991; Matos, *et al.* 1998; Quagio-Grassiotto, *et al.* 2001 b, 2003) as well as in some other teleosts (Jamieson, 1991; Mattei, 1991; Gwo and Gwo, 1993; Gwo, *et al.* 1993, 1994 b; Gwo, 1995; Shahin, 2006 a,c). Conical and elongate form of the nucleus has been described in *C. auratus* (Shahin, 2006 b) and in several characids (Mattei, *et al.* 1995; Burns, *et al.* 1998; Pecio and Rafiński, 1999). In addition, neither nuclear elongation in the spermatozoon nor structures used to attach the flagellum to the nucleus during spermiogenesis are found in *A. dentex*. In other teleosts, however, nuclear elongation has been described in many teleosts (Grier, 1973; Mattei and Mattei, 1974; Todd, 1976; Jones and Butler, 1988; Lahnsteiner and Patzner, 1990; Burns, *et al.* 1998; Pecio and Rafiński, 1999). Moreover, several structures that connect the flagellum with the nucleus have been reported. In *O. mykiss* (Billard, 1983) and eels (Mattei and Mattei, 1974; Todd, 1976; Gwo, *et al.* 1992), there is a structure called flagellar rootlet. In *Oryzias laptipes* (Grier, 1976), *T. thymallus* (Lahnsteiner, *et al.* 1991), *P. flesus* (Jones and Butler, 1988), there is an electron-dense material. Nonetheless, there are bundles of microtubules in *Gambusia affinis* (Grier, 1975).

In spermatozoa of *A. dentex*, there are intercentriolar connections in the form of osmiophilic filaments that arise from the triplets of the proximal centriole and connect it with the distal one. A similar structure has been described also in some species (Billard, 1983; Jones and Butler, 1988; Lahnsteiner, *et al.* 1991; Gwo, 1989; Gwo and Arnold, 1992; Gwo and Gwo, 1993; Gwo, *et al.* 1993; Gwo, *et al.* 1994 a; Gwo, *et al.* 1994 b; Gwo, 1995; Gwo, *et al.* 1995). Moreover, In *A. dentex*, there are two fibrous bodies, which consist of osmiophilic disks alternating with lighter material and lie perpendicularly above the proximal centriole in the upper part of nuclear fossa. These two dense bodies give rise to two short electron-dense fibers, which connect them together with the proximal centriole and anchor the latter to the nucleus. Similar findings have been described in the salmon *O. m. formosanus* by Gwo, *et al.* (1996) who

mentioned that there is a fibrous body, consisting of osmiophilic disks alternating with lighter material, adhering to the two centrioles laterally and linking them together while also linking the proximal centriole with dense bodies in the nuclear fossa. However, in the gadiform *M. merluccius* (Medina, *et al.* 2003), the proximal centriole is linked to the nuclear surface by granular material. In addition, nine radial fibers project from the basal body of the distal centriole triplets and contact the plasma membrane at the caudal part of the centriole. A structure called the intercentriolar lamellate body has been observed in the poeciliids *P. latipinna* (Grier, 1973) and *G. affinis* (Grier, 1975). This structure appears during the development of spermatids to form an electron-dense cap on the proximal end of the basal body and then disappears during the final stage of spermiogenesis. In addition, a connecting piece has been described in *A. japonica* (Gwo, *et al.* 1992). The morphology of the intercentriolar connection is suggested to have a phylogenetic importance among fish (Grier, 1976).

In *A. dentex* there is a striated basal foot attaches the basal body to the nucleus and alar sheets radiate from the basal body triplets and joins it to the plasma membrane. The same structures have been observed in *E. malabaricus* and *P. leopardus* (Gwo, *et al.* 1994 b) and *A. latus* (Gwo, 1995). However, alar sheets have been described in *O. m. formosanus* (Gwo, *et al.* 1996). As described by Gwo (1995), it is probable that both the alar sheets and basal foot function are in the attachment and stabilization of the tail, thereby enabling the centriole to withstand the torque generated by the movement of the flagellum. The occurrence of the basal foot has also been reported in the catfish *C. auratus* and *S. schall* (Shahin, 2006 b,c).

Vesicles, which are commonly shown with more or less regularity in the midpiece of Characiformes spermatozoa (for details, see Quagio-Grassiotto, *et al.* 2003) as well as in *M. electricus* and *C. auratus* (Shahin, 2006 a,b), are entirely absent in *A. dentex*. In addition, the "lattice tubule" described by Mattei *et al.* (1995) in *C.* sp. or the membranous compartment found in the initial region of the flagellum of some Characiformes (Quagio-Grassiotto, *et al.* 2003) as well as some Cypriniformes (Baccetti, *et al.* 1984; Kim, *et al.* 1998; Lee and Kim, 1998) and also the flagellar lateral fins or intratubular differentiations occurring in some teleosts (Mattei, 1988, 1991; Jamieson, 1991) are not found in *A. dentex* examined herein. Similar findings have been reported in many other species of Characiformes for which data are available (for review of species, see Quagio-Grassiotto, *et al.* 2003).

According to the hypothesis of Mattei (1970, 1988), spermatozoa of *A. dentex* seem to be of the primitive type, but they have some peculiar structures that are not previously mentioned in any of the characiform species examined up to date, one of which is the peculiar notch in the nucleus. This structure is observed in *A. schlegeli* (Gwo and Gwo, 1993; Gwo, *et al.* 1993) and *A. latus* (Gwo; 1995) and seems to be found also in some of the curimatid species, but not described by Quagio-Grassiotto, *et al.* (2003). The function and nature of this structure and origins of the nuclear notch and electron-dense material are still obscure. Presumably, the centrioles play some role in the formation of these structures (Gwo and Gwo, 1993). The other structures are the presence of two fibrous bodies, which occupy the upper part of the nuclear fossa and connect the proximal centriole with the nucleus, and the basal foot and alar sheets that attach the basal body to the nucleus and plasma membrane.

The general features of spermiogenesis of *A. dentex* are considerably similar to those of Ostariophysi with external fertilization, which includes Cypriniformes (Baccetti, *et al.* 1984), Characiformes (Jamieson , 1991; Matos, *et al.* 1993; Aires, 1998; Burns, *et al.* 1998; Negrao, 1999; Romagosa, *et al.* 1999; Quagio-Grassiotto, *et al.* 2001 b, 2003), Siluriformes (Poirier and Nicholson, 1982; Maggese, *et al.* 1984; Jamieson, 1991; Mattei, 1991; Quagio-Grassiotto and Carvalho, 2000; Quagio-Grassiotto, *et al.* 2001 a, Shahin, 2006 a,b,c) and Gonorynchiformes (Gwo, *et al.* 1995). Moreover, *A. dentex* spermatozoon has some of the characteristics of Perciformes (Gwo and Gwo, 1993; Gwo, 1995; Gwo, *et al.* 1993, 1994 b) and Salmoniformes (Gwo, *et al.* 1996). Therefore, it could be concluded that the ultrastructural features of spermiogenesis and spermatozoa of *A. dentex* are synapomorphy of Ostariophysi, Perciformes, and Salmoniformes. It is difficult to understand the significance of the ultrastructural characteristics of *A. dentex* spermatozoa in relation to other species of Characiformes because of the lack of data about spermatozoa from other families of this order. On the other hand, the similarity of some morphological features of *A. dentex* spermatozoa to those of Cypriniformes supports the hypothesis that the Cypriniformes are the sister-group of the Otophysi, Characiformes and Siluriformes (Fink and Fink, 1996; Shahin, 1997, 1999).

Acknowledgments

The author would like to thank Prof. Dr.

emeritus M. A. Ramadan for reading the manuscript, Dr. H. A. El Azm, Electron Microscopy Unit in Minia University Lab for Microanalysis, for technical assistance, and the Fisherman Mr. M. Hussein for providing the fish.

References

Aires, E.D. (1998) Características morfológicas e histofisiológicas da via espermática da Piracanjuba, Brycon orbignyanus (Pisces, Teleostei). Ph.D. Dissertation, Universidade Estadual Paulista, Botucatu, 100 P (unpublished).

Andrade, R.F., Bazzoli, N., Rizzo, E., and Sato, Y. (2001) Continuous gametogenesis in the neotropical freshwater teleost, *Bryconops affinis* (Pisces: Characidae). Tissue Cell **33:** 524-532.

Baccetti, B. (1985) Evolution of the sperm cell, *In:* **Metz, C.B. and Monroy, A. (eds.).** Biology of fertilization, Vol 2. Academic Press, Orlando, 3-58 pp.

Baccetti, B. (1986) Evolutionary trends in sperm structure. Comp. Biochem. Physiol. **85:** 29-36.

Baccetti, B., Burrini, A.G., Callaini, G., Gibertini, G., Mazzini, M., and Zerunian, S. (1984) Fish germinal cells. I. Comparative spermatology of seven cyprinid species. Gamete Res. **10:** 373-396.

Billard, R. (1970) Ultrastructure comparee de spermatozoides de quelques poissons teleosteens, *In:* **Baccetti, B. (ed.).** Comparative spermatology. Academic Press, New York, 71-79 pp.

Billard, R. (1983) Ultrastructure of trout spermatozoa: Changes after dilution and deep freezing. Cell Tissue Res. **228:** 205-218.

Billard, R. (1986) Spermatogenesis and spermatology of some teleost fish species. Reprod. Nutr. Dev. **26:** 877-920.

Brusle, S. (1981) Ultrastructure of spermatogenesis in *Liza aurara* Risso, 1810 (Teleostei, Muglidae). Cell Tissue Res. **217:** 415-424.

Burns, J.R., Weitzman, S.H., Lange, K.R., and Malabarba, L.R. (1998) Sperm ultrastructure in characid fishes (Teleostei: Ostariophysi), *In:* **Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena, Z.M. and Lucena, C.A.S. (eds.).** Phylogeny and classification of Neotropical fishes. Edipucrs, Porto Alegre, 235-244 pp.

Fink, S.V. and Fink, W.L. (1996) Interrelationships of Ostariophysan fishes, *In:* **Stiassny, M.L.J., Parenti, L.R. and Johnson, G.D. (eds.).** Interrelationships of fishes. Academic Press, San Diego, 209-249 pp.

Franzen, A. (1970) Phylogenetic aspects of the morphology of spermatozoa and spermatogenesis, *In:* **Bacaetti, B. (ed.).** Comparative spermatology. Academic Press, New York, 26-46 pp.

Fribourgh, J.H., McClendon, D.E., and Soloff, B.L. (1970) Ultrastructure of the goldfish, *Carassius auratus* (Cyprinidae) spermatozoa. Copeia **2:** 274-279.

Gardiner, D.M. (1978) Fine structure of the spermatozoon of the viviparous teleost, *Cymatogaster aggregata*. J. Fish. Biol. **13:** 435-438.

Ginzburg, A.S. (1968) Fertilization in fishes and the problem of polyspermy, 1st ed. Academy of Science, Moscow, 358 p.

Grier, H.J. (1973) Ultrastructure of the testis in the teleost *Poecilia latipinna* spermatogenesis with reference to the intercentriolar lamellated body. J. Ultrastruct. *Res.* **45:** 82-92.

Grier, H.J. (1975) Spermatogenesis in the teleost *Gambusia affinis* with particular reference to the role played by microtubules. Cell Tissue Res. **165:** 89-102.

Grier, H.J. (1976) Sperm development in teleost *Oryzias latipes*. Cell Tissue Res. **168:** 419-431.

Grier, H.J., Fitzsimons, J.M., and Linton, J.R. (1978) Structure and ultrastructure of the testis and sperm formation in goodeid teleosts. J. Morphol. **156:** 419-438.

Guan, T.L. and Afzelius, B.A. (1991) The spermatozoon of the Chinese bitterling, *Rhodeus sericeus sinensis* (Cyprinidae, Teleostei). J. Submicrosc. Cytol. Pathol. **23:** 351-356.

Gwo, J.C. (1989) *Cryopreservation of Atlantic croaker spermatozoa:* Optimization of procedures, evaluation of morphological changes and sssessment of motility. Ph.D. Dissertation, Texas A and M University, College Station, Texas, 98 P (unpublished).

Gwo, J.C. (1995) Spermatozoan ultrastructure of the teleost fish *Acanthopagrus latus* (Perciformes: Sparidae) with special reference to the basal body. J. Submicrosc. Cytol. Pathol. **27:** 391-396.

Gwo, J.C. and Arnold, C.R. (1992) Cryopreservation of Atlantic croaker spermatozoa: Evaluation of morphological changes. J. Exp. Zool. **264:** 444-453.

Gwo, J.C. and Gwo, H.H. (1993) Spermatogenesis in the black porgy (Teleostei, Perciformes, Sparidae). Mol. Reprod. Dev. **36:** 75-83.

Gwo, J.C., Gwo, H.H.. and Chang, S.L. (1992) The spermatozoon of the Japanese eel, *Anguilla japonica* (Teleostei, Anguilliformes, Anguillidae). J. Submicrosc. Cytol. Pathol. **24:** 571-574.

Gwo, J.C., Gwo, H.H., and Chang, S.L. (1993) The ultrastructure of *Acanthopagrus schlegeli* spermatozoon. J. Morphol. **216:** 29-33.

Gwo, J.C., Lin, X.W., Kao, Y.S., and Chang, H.H. (1994a) The ultrastructure of ayu, *Plecoglossus altivelis*, spermatozoon (Teleostei, Salmoniformes, Plecoglossidae). J. Submicrosc. Cytol. Pathol. **26:** 467-472.

Gwo, J.C., Gwo, H.H., Kao, Y.S., Lin, B.H. , and Shih, H. (1994b) Spermatozoan ultrastructure of two species of grouper *Epinephelus malabaricus* and *Plectropomus leopardus* (Teleostei, Perciformes, Serranidae) from Taiwan. J. Submicrosc. Cytol. Pathol. **26:** 131-136.

Gwo, J.C., Lin, X.W., Kao, Y.S., Chang, H.H., and Su, M.S. (1995) The ultrastructure of milkfish, *Chanos chanos* (Forsskål), spermatozoon (Teleostei, Gonorynchiformes, Chanidae). J. Submicrosc. Cytol. Pathol. **27:** 99-104.

Gwo, J.C., Lin, X.W., Gwo, H.H., Wu, H.C. , and Lin, P.W. (1996) The ultrastructure of Formosan landlocked salmon, *Oncorhynchus masou formosanus*, spermatozoon (Teleostei, salmoniformes, salmonidae). J. Submicrosc. Cytol. Pathol. **28:** 33-40.

Iatrou, K. and Dixon, G.H. (1978) Protamine messenger RNA: Its life history during spermatogenesis in rainbow trout. Fed. Proc. **37:** 2526-2533.

Jamieson, B.G.M. (1991) Fish evolution and systematics: Evidence from spermatozoa. Cambridge University Press, Cambridge, 320 p.

Jamieson, B.G.M. and Grier, H.J. (1993) Influences of phylogenetic position and fertilization biology on spermatozoal ultrastructure exemplified by exocoetoid and poeciliid fish. Hydrobiologia **271:** 11-25.

Jones, P.R. and Butler, R.D. (1988) Spermatozoon ultrastructure of *Platichthys flesus*. J. Ultrastruct. Mol. Struct. Res. **98:** 71-82.

Kim, K.H., Kwon, A.S., and Lee, Y.H. (1998) Spermatozoal ultrastructure and phylogenetic relationship of the subfamily Gobioninae (Cyprinidae) 2. Ultrastructure of spermatozoa in the Korean gudgean, *Squalidus chankaensis tsuchigae*. Korean J. Limnol. **31:** 159-164.

Lahnsteiner, F. and Patzner, R.A. (1990) Spermiogenesis and structure of mature spermatozoa in bleniid fishes (Pisces, Blenniidae). J. Submicrosc. Cytol. Pathol. **22:** 565-576.

Lahnsteiner, F., Patzner, R.A., and Weisman, T. (1991) Fine structure of spermatozoa of the grayling (*Thymallus thymallus*, Pisces, Teleostei). J. Submicrosc. Cytol. Pathol.**23:** 373-377.

Lee, Y.H. and Kim, K.H. (1998) Ultrastructure of spermatozoa in *Pungtungia herzi*. Dev. Reprod. **2:** 141-148.

Lou, Y.H. and Takahashi, H. (1989) Spermatogenesis in the Nile tilapia *Oreochromis niloticus* with notes on a unique pattern of nuclear chromatin condensation. J. Morphol. **200:** 321-330.

Magalhães, A.L.B. de. (1998) Gametogénesis e reproducão de *Galeocharax knerii* (Steindachner, 1879) (Pisces, Characidae) nos reservatórios de Furnas e Itumbiara: estudo biométrico, histológico e ultra-estrutural. M.Sc. Dissertation, Universidade Federal de Minas Gerais, Belo Horizonte, 122 P (unpublished).

Maggese, M.C., Cukier, M., and Cussac, V.E. (1984) Morphological changes, fertilizing ability and motility of *Rhamdia sapo* (Pisces, Pimelodidae). Sperm induced by media of different salinities. Revista Brasil. Biol. **44:** 541-546.

Mansour, N., Lahnsteiner, F. , and Patzner, C.A. (2002) The spermatozoon of the African catfish: fine structure, mobility, viability and behavior in seminal vesicle secretion. J. Fish Biol. **60:** 545-560.

Matos, E., Matos, P., Oliveria, E., and Azevedo, C. (1993) Ultraestrutura do espermatozóide do pacu, *Metynnis maculates* Kner, 1860 (Pisces, Teleostei) do rio Amazonas. Revista Brasil. Cién. Morfol. **10:** 7-10.

Matos, E, Matos, P., Santos, M.N.S., and

Azevedo, C. (1998) Aspectos morfológicos e ultraestruturais do espermatozóide de *Curimata inornata* Vari, 1989 (Pisces, Teleostei) do rio Amazonas. Acta Amaz. **28:** 449-453.

Matos, E., Matos, P., Corral, L., and Azevedo, C. (2000) Estrutura fina do espermatozóide de *Acestrorhyncus falcatus* Bloch (Teleostei, Characidae) da região norte do Brasil. Revista Brasil. Zool. **17:** 747-752.

Mattei, C. (1970) Spermiogeneses comparee des poisons, *In:* **Baccetti, B. (ed.).** Comparative spermatology. Academic Press, New York, 57-69 pp.

Mattei, X. (1988) The flagellar apparatus of spermatozoa in fish. Ultrastructure and evolution. Biol. Cell. **63:** 151-158.

Mattei, X. (1991) Spermatozoon ultrastructure and its systematic implications in fishes. Can. J. Zool. **69:** 3038-3055.

Mattei, C. and Mattei, X. (1974) Spermiogenesis and spermatozoa of the elopomorpha (teleost fish), *In:* **Afzelius, B.A. (ed.).** The Functional anatomy of the spermatozoon. Pergamon Press, New York, 211-221 pp.

Mattei, X., Marchand, B., and Thiaw, O.T. (1995) Unusual midpiece in the spermatozoon of the teleost fish, *Citharinus* sp. J. Submicrosc. Cytol. Pathol. **27:** 189-191.

Medina, A., Megina, C., Abascal, F.J., and Calzada, A. (2003) The sperm ultrastructure of *Merluccius merluccius* (Teleostei, Gadiformes): phylogenetic considerations. Acta Zool. (Stockholm) **84:** 131-137.

Negrão, J.N.C. (1999) Aspectos ultraestruturais da espermatog-nese da traira, *Hoplias malabaricus (Pisces, Erythrinidae).* Instituto de Biociûncias, Universidade Estadual Paulista, Botucatu, 110 P (unpublished).

Pecio, A. and Rafiński, J. (1999) Spermiogenesis in *Mimagomiates barberi* (Teleostei: Ostariophysi: Characidae), an oviparous, internally fertilizing fish. Acta. Zool. (Stockholm) **80:** 35-45.

Poirier, G.R. and Nicholson, N. (1982) Fine structure of the testicular spermatozoa from the channel catfish, *Ictalurus punctatus*. J. Ultrastruct. Res. **80:** 104-110.

Quagio-Grassiotto, I. and Carvalho, E.D. (2000) Ultrastructure of *Sorubim lima* (Teleostei, Siluriformes, Pimelodidae) spermiogenesis. J. Submicrosc. Cytol. Pathol. **32:** 629-633.

Quagio-Grassiotto, I., Oliveira, C., and Gosztonyi, A.E. (2001a) The ultrastructure of spermiogen-esis and spermatozoa in *Diplomystes mesembrinus*. J. Fish Biol. **58:** 1623-1632.

Quagio-Grassiotto, I., Negrão, J.N.C., Carvalho, E.D., and Foresti, F. (2001b) Ultrastructure of spermatogenic cells and spermatozoa in *Hoplias malabaricus* (Teleostei, Characiformes, Erythrinidae). J. Fish Biol. **59:** 1494-1502.

Quagio-Grassiotto, I., Gameiro, M.C., Schneider, T., Malabarba, L.R., and Oliveira, C. 2003. Spermiogenesis and spermatozoa ultrastructure in five species of the Curimatidae with some considerations on spermatozoal ultrastructure in the Characiformes. Neotrop. Ichthyol. **1:** 35- 45.

Romagosa, E., Narahara, M.Y., Borella, M.I., Barreira, S.F., and Fenerich-Verani, N. (1999) Ultrastructure of the germ cells in the testis of matrinxã, *Brycon cephalus* (Teleostei, Characidae). Tissue Cell **31:** 540-544.

Shahin, A.A.B. (1997) The caudal skeleton of some cypriniform fishes in Egypt and its bearing on their taxonomic relationships with Siluriformes. J. Union Arab Biol. **7(A):** 185-216.

Shahin, A.A.B. (1999) Phylogenetic relationship between the characid Nile fish *Alestes dentex*, cyprinids *Barbus bynni*, *Labeo niloticus*, and the introduced grass carp *Ctenopharyngodon idella* elucidated by protein electrophoresis. J. Egypt. Ger. Soc. Zool. **29 (B):** 21-42.

Shahin, A.A.B. (2006a) Semicystic spermatogenesis and biflagellate spermatozoon ultrastructure in the Nile electric catfish *Malapterurus electricus* (Teleostei: Siluriformes: Malapteruridae). Acta Zool. (Stockholm) **87:** 215-227.

Shahin, A.A.B. (2006b) A noval type of spermiogenesis in the Nile catfish *Chrysichthys auratus* (Siluriformes: Bagridae) in Egypt, with description of spermatozoon ultrastructure. Zool. In the Middle East: In publication.

Shahin, A.A.B. (2006c) Spermatogenesis and spermatozoon ultrastructure in the Nile catfish *Synodontis schall* (Teleostei: Siluriformes: Mochokidae) in Egypt. Acta Zool. (Stockholm): In publication.

Silveira, H., Rodrigues, P., and Azevedo, C. (1990) Fine structure of the spermatogenesis of *Blennius pholis* (Pisces, Blenniidae). J. Submicrosc. Cytol. Pathol. **22:** 103-108.

Sprando, R.L., Heidinger, R.C., and Russell, L.D. (1988) Spermiogenesis in the bluegill (*Lepomis macrochirus*): a study of cytoplasmic events including cell volume changes and cytoplasmic elimination. J. Morphol. **198:** 165-177.

Stein, H. (1981) Licht- und elektronenoptische Untersuchungen an der Spermatozoen verschiedener Susswasserknochenfische (Teleostei). Z. Angew. Zool. **68:** 183-198.

Thiaw, O.T., Thiaw, D., and Mattei, X. (1990) Extension of proximal centriole in a teleost fish spermatozoon. J. Submicrosc. Cytol. Pathol. **22:** 357-360.

Todd, P.R. (1976) Ultrastructure of spermatozoa and spermatogenesis in New Zealand freshwater eels (Anguillidae). Cell Tissue Res. **171:** 221-232.

Vari, R.P. (1989) A phylogenetic study of the Neotropical characiform family Curimatidae (Pisces: Ostariophysi). Smithsonian Contr. Zool. **471:** 1-71.

Veríssimo-Silveira, R. (2003) Ciclo reproductivo e cinética da espermatogénese do dourado *(Salminus maxillosus, 1849).* M.Sc. Dissertation, Universidade Estadual Paulista, Jaboticabal, 60 P (unpublished).

Zaiden, S.F. (2000) Morfologia gonadal e metabolismo energético da piraputanga Brycon hilarii *(Cuvier e Valenciennes)* (Pisces, Characidae) em cativeiro, durante o ciclo reproductivo annual. Ph.D Dissertation, Universidade Estadual Paulista, Jaboticabal, 152 P (unpublished).

Ref: 2357
Received: 21/04/2006
In revised form: 26/05/2006