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Male reproductive system in the South American catfish *Conorhynchus conirostris*

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The testes of the catfish *Conorhynchus conirostris* ($n = 67$) from the São Francisco River, Minas Gerais, Brazil were of the fringed type, similar to those of some Pimelodidae. The germ, Sertoli and Leydig cells showed characteristics which are general for all vertebrates although the spermatozoa had a peculiar morphology, with an ovoid head without an acrosome, inverted U-shaped nucleus, a short midpiece and a long tail, typical of teleosts showing external fertilization. The spermatic duct and genital papilla performed a secretory function.

The Siluriformes form a diverse group of marine and freshwater fishes with about 2000 species (Nelson, 1994). Siluriformes have a varied testicular morphophysiology, with the testes being either spermatogenic only, possessing a spermatogenic cranial region and a secretory caudal region, or showing accessory seminal vesicles (Loir *et al*., 1989; Santos *et al*., 2001).

Testicular morphology of Siluriformes is quite variable. The testes may be elongated showing digitiform projections, or fringes (most families of Siluriformes) or may be elongated, with no fringes or projections as in Helogeneidae and Ariidae (Loir *et al*., 1989).

In the Siluriformes with external fertilization, studies of spermatozoon ultrastructure are available for only a few species: *Ictalurus punctatus* (Rafinesque) (Poirier & Nicholson, 1982), *Rhamdia sapo* (Valenciennes) (Maggese *et al*., 1984), *Heteropneustes fossilis* (Bloch) (Nath & Chand, 1998), *Sorubim lima* (Bloch & Schneider) (Quagio-Grassiotto & Carvalho, 1999) and *Iheringichthys labrosus* (Lütken) (Santos *et al*., 2001). Even though the spermatic duct and genital papilla are known to play an important role in the release of spermatozoa and in the dynamics of fertilization, studies on these structures are rare, particularly, in Siluriformes (Rasotto & Shapiro, 1998).

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The pirá catfish *Conorhynchus conirostris* (Valenciennes) an endemic fish in the São Francisco River basin, belongs to the order Siluriformes, family Pimelodidae, and may reach up to 100 cm total length (L_T) and 13 kg in body mass (Sato, 1999). It is a migratory fish of commercial importance and is included in the list of the species threatened with extinction in this basin (Lins *et al.*, 1997).

In order to study the testicular morphophysiology and the ultrastructure of the spermatogenic cells, spermatic duct and genital papilla, specimens of pirá were obtained from a commercial fishery from December 1998 to January 2000 in the São Francisco River, near Pirapora, Minas Gerais, Brazil (17°20′45″ S; 44°56′55″ W). Males were weighed (M, g) and killed by decapitation and their reproductive systems ($n = 10$) were removed, weighed (M_T, g) and fixed in 10% formalin for anatomical study. Fragments ($n = 67$) of testes, spermatic duct and genital papilla were fixed in Bouin's liquid, embedded in paraffin or glycol methacrylate, then cut into 3–5 μm sections, and stained with haematoxylin-eosin or 1% toluidin blue-sodium borate. Fragments ($n = 10$) of testes, spermatic duct and genital papilla were subjected to classical histochemical techniques for the detection of carbohydrates and proteins (Pearse, 1985): Periodic Acid-Schiff (PAS), alcian blue pH 2·5 and 0·5, and Ninhydrin-Schiff. For the ultrastructural study, fragments of the testes, spermatic duct and genital papilla of five specimens were fixed in 2·5% glutaraldehyde in phosphate buffer (0·1 M, pH 7·3) for 12 h at 4° C, then post-fixed in 1% osmium tetroxide in phosphate buffer (0·1 M) for 2 h, and embedded in Epon. Ultrathin sections were cut with a diamond knife in an ultramicrotome SORVALL MT2-B and stained with uranyl acetate and lead citrate and examined under a ZEISS EM-10 transmission electron microscope.

The testes of *C. conirostris* had a small volume and gonado-somatic index (I_G, $I_G = 100\ M_T M^{-1}$) always <0·5% of body mass, and were paired organs with digitiform projections, or fringes, along their entire length [Fig. 1(a)]. They were located in the coelomic cavity and were dorsally supported by the mesorchia. The fringes communicated with the spermatic duct, located in the central portion of the testis [Fig. 1(b)]. The spermatic ducts of the right and left testes were joined at their caudal portions, forming the common spermatic duct, extending to the genital papilla, situated caudally to the anal opening. The genital papilla was conic, without fringes and lined by simple columnar epithelium. The testes were surrounded by a tunica albuginea of connective tissue, that emitted septa to the interior of the organ, delimiting seminiferous tubules made up of spermatocysts [Fig. 1(c)]. Within each spermatocyst, germ cells were at the same stage of development [Fig. 1(d)]. The walls of the spermatocysts were formed by cytoplasmic processes of Sertoli cells.

Despite their fringed morphology, the testes of *C. conirostris* are similar histologically to most teleosts, with spermatogonia distributed along the seminiferous tubules and spermatogenic activity along their entire length; that is, an unrestricted testis type as described by Grier (1981). In contrast to *I. labrosus* (Santos *et al.*, 2001), the caudal region of the testes of *C. conirostris* is not secretory. In the present study, seminal vesicles or accessory glandular structures were not observed in the reproductive system as in some Siluriforms (Loir *et al.*, 1989).

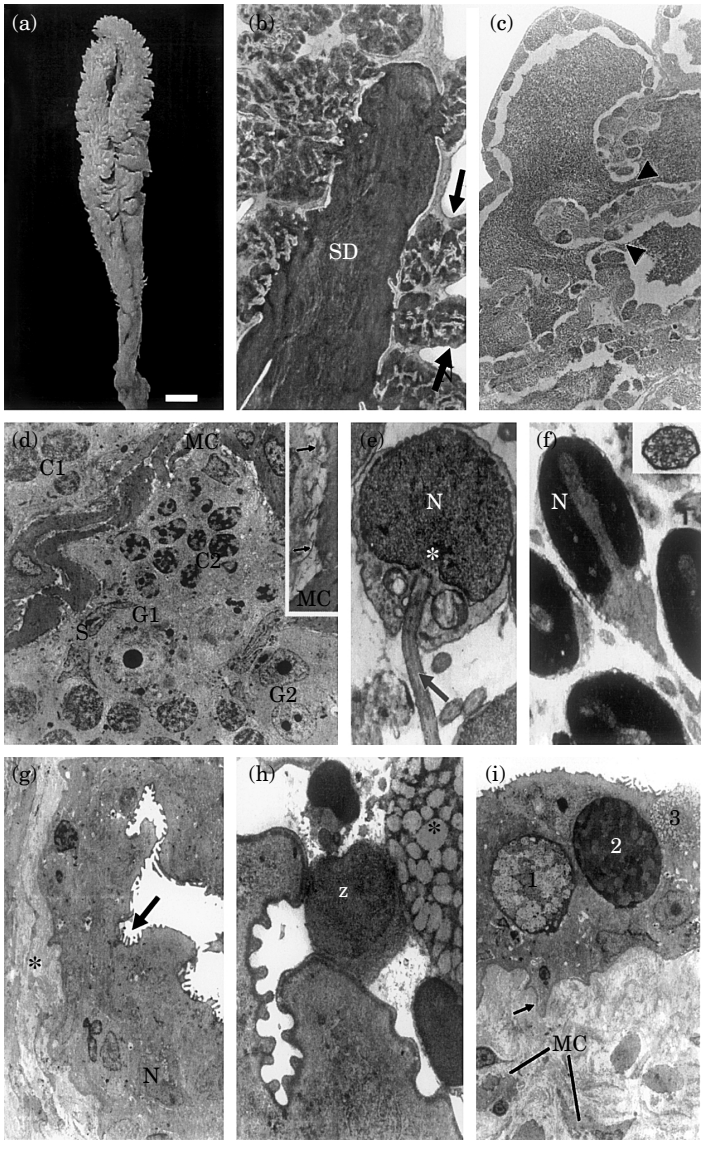

FIG. 1. Organization of male reproductive system of *C. conirostris*. (a) Gross morphology of fringed testis. x1·3. Scale bar = 1 cm. (b) Cross-section of testis showing digitiform projections (→) and spermatic duct (SD) filled with spermatozoa. Haematoxylin–eosin; x50. (c) Testicular fringe showing anastomosis (▶) between seminiferous tubules. Toluidine blue-sodium borate; x100. (d) Ultrastructure of germ cells: primary spermatogonia (G1), secondary spermatogonia (G2), primary spermatocytes (C1) and secondary spermatocytes (C2); MC, myoid cells; S, Sertoli cells. x2400. Inset: collagen fibrils (→) attached to plasma membrane of the myoid cells (MC). x5000. (e) Spermatids with condensed nucleus (N), implantation fossa (*) and flagellum (→). x14 250. (f) Longitudinal section of spermatozoa with inverted U-shaped nucleus (N) in the head, and short midpiece. x12 750. Insert: ultrastructure of flagellum showing microtubules in 9 + 2 axonemal arrangement. x26 600. (g) Ultrastructure of spermatic duct showing epithelial cells with microvilli (→) and nucleus (N) supported by a lamina propria (*) with collagen fibrils and myoid cells. x1615. (h) Spermatozoa (Z) and secretory vesicles (*) in the spermatic duct. x11.000. (i) Ultrastructure of genital papilla with mucous cells at different functional stages (1, 2 and 3); →, basement membrane; MC, myoid cells. x1715.

In *C. conirostris*, the myoid cells had an elongated shape with a fusiform nucleus, they were arranged in discontinuous, concentric layers around the seminiferous tubules [Fig. 1(d)] and showed electron-dense regions on the inner side of the plasma membrane forming macular junction contacts with other cell types in the testis. In addition, collagen fibrils appeared to attach to the plasma membrane of the myoid cells [Fig. 1(d); inset], similar to those described in *Esox lucius* L. and *Esox niger* Lesueur (Grier *et al.*, 1989). The myoid cells contained a cytoplasm rich in microfibrils and they may contribute to the spermiation process as observed by Yaron (1995) and Santos *et al.* (2001).

During spermiogenesis, the early spermatids, with a scant cytoplasm and a nucleus in condensation process [Fig. 1(e)], gradually differentiated into spermatozoa, with an ovoid head, no acrosome, a short midpiece and a long flagellum with a $9+2$ axonemal arrangement [Fig. 1(f); inset]. In the head, condensed chromatin with an inverted U- or horseshoe-shape, and a deep penetration of the nuclear fossa were visible [Fig. 1(f)]. The single flagellum had scant cytoplasm surrounded by a cytoplasmic membrane, without lateral fins. These characteristics are typical of primitive spermatozoa of fishes with external fertilization (Billard, 1969). According to the classification proposed by Jamieson (1991), the sperm morphology of *C. conirostris* corresponds to the simple type called aquasperm, which has a round or ovoid head and short midpiece containing few mitochondria. In addition to these morphological features that are common to the spermatozoa of many teleosts, inverted U- or horseshoe-shaped chromatin and a deep nuclear fossa were also observed in the siluriform *Heteropneustes fossilis* (Bloch) (Nath & Chand, 1998) and in the tetraodontiform *Balistes forcipatus* (Gmelin) (Jamieson, 1991). This type of nuclear fossa, which penetrates almost to the tip of the nucleus constitutes a divergence from the common teleost sperm ultrastructure, as has been reported by Quagio-Grassiotto *et al.* (2001). The spermatozoa of *C. conirostris* had a single flagellum as in most species of teleosts but some were biflagellated, as in *R. sapo* (Maggese *et al.*, 1984). The pirá sperm tail does not have flagellar fins as is common in sperm of others siluroids and gymnotoids, providing weak support for a close relaionship between Siluriformes, Cypriniformes and Characiformes (Jamieson, 1991). The acrosome-less head in the spermatozoa of *C. conirostris* is a common characteristic in teleosts, related to external fertilization and the presence of micropyle in the eggs (Jamieson, 1991).

During spermiation, the spermatocysts released mature sperm and the seminiferous tubules had compartments that looped at the testis periphery forming a continuously anastomosing tubular system [Fig. 1(c)], connecting to the spermatic duct, similar to the observations of Grier (1993). The wall of the spermatic duct was lined by secretory prismatic cells with microvilli and a lamina propria made up of myoid cells and collagen fibrils [Fig. 1(g)]. The lumen of the common spermatic duct may have contained residual spermatozoa after spermiation and the cells lining the lumen may have contained secretory vesicles [Fig. 1(h)]. According to Billard & Takashima (1983) and Rasotto & Shapiro (1998), the spermatic duct may participate in the transport of spermatozoa, in the secretion of substances that form the seminal fluid, and in the reabsortion of residual spermatoza.

The sperm are released into the spermatic duct upon spermiation and subsequently pass through the sperm duct, which itself passes through the genital papilla whose wall consisted of simple columnar epithelium with microvilli and mucous cells at different functional stages [Fig. 1(i)]. Myoid cells and collagen fibrils were abundant in the lamina propria of the genital papilla [Fig. 1(i)]. Positive reactions to PAS, Ninhydrin-Schiff and alcian blue (pH 2·5 and 0·5) indicated that the mucous cells contained neutral glycoproteins associated with carboxylated acid glycoconjugates. Histological and histochemistry evidence in the present study suggest that mucous cells may be involved in secretory activity and myoid contractile cells could participate in the release of gametes, as reported in other species (Rasotto & Shapiro, 1998; Richtarski & Patzner, 2000). Besides genital papilla, positive reactions to PAS were also observed in the basal membrane of the seminiferous tubules, in the basal membrane situated between connective tunica and the cysts, and in secretions presented in the lumen of the seminiferous tubules.

The fringed testicular morphology of *C. conirostris* without seminal vesicles or accessory glandular structures is similar to those observed in the pimelodid *Pseudoplatystoma corruscans* (Spix & Agassiz) (Brito & Bazzoli, 2003).

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