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Testis structure, spermatogonial niche and Sertoli cell efficiency in Neotropical fish

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ABSTRACT

Neotropical icthyofauna represents one of the most diverse and extreme ecosystems in the world. Likewise, reproduction showed enormous diversity with different reproductive systems, modes and behavior. On the other hand, information on Neotropical fish species, in particular on male reproductive physiology is restricted to few species. This mini-review aimed to compile the existing information on spermatogenesis of Neotropical teleosts focusing on testis structure, spermatogonial niche and Sertoli cell efficiency. The first topic covers the histological analysis of the testicular structure, showing a conserved testicular pattern in relation to the phylogenetic position: basal species present anastomosing tubular testis (e.g. *Astyanax altiparanae*, *Conorhynchos conirostris, Pimelodus maculatus, Lophiosilurus alexandri, Rhinelepis aspera*, among others), while derived teleosts showed lobular testis (e.g. *Cichlasoma dimerus*, *Cichla kelberi, Odontesthes bonariensis, Synbranchus marmoratus* and others). Next to testicular structure, existing data showed that type A undifferentiated spermatogonia (A_{und}) is differentially distributed among the Neotropical species. A_{und} can be restricted at the blind-end of the germinal compartment (*O. bonariensis)*, or spread along the germinal epithelium (*A. altiparanae*), or even distributed along the germinal epithelium but concentrated at the blind-end (*C. kelberi* and *C. intermedia*). Moreover, recent studies in *A. altiparanae* have demonstrated that within the germinal compartment, A_{und} have a preferential distribution in areas neighboring the interstitial compartment – the spermatogonial niche. The proximity with the interstitium suggests that interstitial cells, such as Leydig cells, are important for A_{und} maintenance in the testis. Finally, this mini-review highlighted Sertoli cell efficiency, showing that a single Sertoli cell can support a higher number of germ cells (80–140 spermatids) in Neotropical species evaluated at the moment (e.g. *A. altiparanae*, *Hoplias malabaricus*, *Poecilia reticulata*, *Serrasalmus spilopleura*, *C. intermedia*). Overall, this review provided basic and functional information on spermatogenesis of Neotropical species. More studies in this field are necessary since Neotropical region is considered one of the hotspot regions to discovery new species providing, therefore, new opportunities to investigate spermatogenesis in fish.

1. Introduction

Considered the most diverse group among vertebrates, fishes comprise around 32.000 species, which represent over than a half of the living vertebrates in the world (Nelson et al., 2016). Part of this diversity is concentrated in the Neotropical realm, where the success of the group can be attributed to different reproductive strategies, ranging from species with intertidal spawning such as the yellow tetra *Astyanax altiparanae* (Orsi et al., 2004) to total spawning as seen in the matrinxã *Brycon orthotaenia* (Gonçalves et al., 2006). Next to the different

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strategies, a vast array of amazing adaptations is seen in fish gonadal structure. Testes are usually paired and elongated organs, as observed in most of the teleosts, or they are formed by several fringes as commonly found in the Neotropical catfishes, such as *Pseudoplatystoma fasciatum* (Batlouni et al., 2006), *P. corruscans* (Brito and Bazzoli, 2003) and *Steindachneridion parahybae* (Honji et al., 2013). Although not common, single testis can also be found, as seen in *Tomeurus gracilis* (Parenti et al., 2010).

Although diverse, the testicular structure is conserved among the species; testes are divided into two compartments separated by a basement membrane: the germinative and the interstitial compartments. In the germinative compartment, spermatogenesis takes places within the germinal epithelium (Grier, 2002; Brown-Peterson et al., 2002; Grier et al., 2016). Although spermatogenic process is highly conserved among vertebrates (Grier et al., 2016), fish spermatogenesis displays some peculiarities. One of these peculiarities is the germ cell development inside of the so-called spermatocysts or cysts, formed when a single, undifferentiated spermatogonia is completely surrounded by Sertoli cells (Schulz et al., 2010).

Spermatogenesis is a stem-cell driven process, which consists of highly coordinated and complex events, where a single undifferentiated spermatogonia pass through numerous species-specific mitotic divisions (Schulz and Nóbrega, 2011a,b), two meiosis and a morphological transformation (differentiation) to originate the final male gamete (spermatozoon). Fish spermatogenesis is directly influenced by abiotic factors such as the water temperature. For example, while higher temperatures would trigger rapid germ cell differentiation, lower temperatures would favor type A spermatogonia renewal, Sertoli and Leydig cell proliferation, and germ cell apoptosis in the Nile tilapia (*Oreochromis niloticus*) (Alvarenga et al., 2009). On the other hand, it has been shown in *A. altiparanae* (Siqueira-Silva et al., 2015) and in the Pejerrey *Odontesthes bonariensis* (Ito et al., 2008) that higher temperatures induce germ cell apoptosis. These differences suggest that spermatogenesis response to environmental change of water temperature is species-specific. Other abiotic factors like photoperiod and salinity can also influence fish spermatogenesis (Strüssmann et al., 2010; Reynalte-tataje et al., 2002; Reid et al., 2013).

As discussed above, despite of the similarities with other vertebrates, fish spermatogenesis exhibited species-specific morpho-physiological peculiarities. Considering that knowledge on spermatogenesis of Neotropical species is restricted to few species, this mini-review aimed to compile the existing information on this subject focusing in testicular germinal epithelium and its components, with special attention to spermatogonia and Sertoli cell. The special attention to Neotropical region is mentioned by Nelson and colleagues (Nelson et al., 2016), which considered one of the hotspot regions in the world for the discovery of new species in the last decade, and also because it is the part of the tropical area that have the vast majority of fish species in the world (Nelson et al., 2016).

2. Testis structure

Testicular structure is quite diverse among Neotropical teleosts. The distintic testicular morphologies can be related to the different reproductive strategies and also the result of evolutionary adaptations to the most diverse aquatic habitats of the Neotropical region. For example, in the protogynous *Synbranchus marmoratus*, testicular structure reflected the reproductive strategies adopted by this species; paired or single testis are derived from primary and secondary males, respectively (Lo Nostro et al., 2003a,b). In this case, the single testis was originated from a single ovary during the sex reversal process of this species (Lo Nostro et al., 2003a,b). Despite of the anatomical differences, most of the species present paired, filiform, and elongated testes that are caudally joint into a spermatic duct (de Siqueira-Silva et al., 2017), such as in the basal teleost *A. altiparanae* (Fig. 1a, b) (Parenti and Grier, 2004).

Microscopically, testes are surrounded by a dense connective tissue, named tunica albuginea, which emits septa dividing the parenchyma into a germinal and interstitial compartments, as illustrated in *A. altiparanae* and *C. kelberi* testes (Siqueira-Silva et al., 2013) (Fig. 2). These two compartments are separated from each other by a basement membrane. The germinal compartment is formed by germ cells in association with somatic Sertoli cells, forming the so-called spermatogenic cysts. The spermatogenic cyst is formed when a single, undifferentiated spermatogonia is completely involved by cytoplasmic extensions of Sertoli cells. The cystic structure is considered the morpho-functional unity of the spermatogenesis in anamniote vertebrates (Schulz et al., 2010; Nóbrega et al., 2009). The interstitial compartment shelters the steroid-producing Leydig cells, peritubular myoid cells, blood vessels, neural and connective tissue elements, including blood cells (erythrocytes, lymphocytes, monocytes, granulocytes, macrophages and histamine positive cells) (Schulz et al., 2010; Nóbrega et al., 2009; Lo Nostro et al., 2004; Chaves-Pozoet al., 2018).

The Leydig cells are the main steroid-producing cells in the testis. They are usually detected by 3β-hydroxysteroid dehydrogenase (3β-HSD) activity and display cytoplasm with a well-developed smooth endoplasmic reticulum (SER) and numerous mitochondria with tubular cristae, as described in the Neotropical species, *S. spilopleura* (Nóbrega and Quagio-Grassiotto, 2007); *S. marmoratus* (Lo Nostro et al., 2004) and *Bryconops affinis* (Andrade et al., 2001). The Leydig cells express receptors for both gonadotropins, Fsh (Follicle-stimulating hormone) and Lh (Luteinizing hormone) which trigger androgen synthesis and release in these cells (Schulz and Nóbrega, 2011a,b).

The myoid or peritubular myoid cells form a discontinuous layer surrounding the basement membrane of the germinal compartment (Schulz et al., 2010). It is believed that myoid cells are the main source of extracellular matrix in the interstitium. Myoid cells are usually found between the connective tissue elements, such as collagen fibers, which can also attach to myoid cell membrane through focal contacts. The myoid cell cytoplasm exhibits contractile structures, such as microfilaments that run parallel to the long axis of the cell and several dense bodies, as described in *S. marmoratus* (Lo Nostro et al., 2004).

The erythrocytes, lymphocytes, monocytes, acidophilic granulocytes and macrophages are the main blood cells detected by transmission electron microscope in the interstitial compartment of *S. marmoratus* (Lo Nostro et al., 2004). Interestingly, the acidophilic granulocytes were also found in the testis of the gilthead seabream *Sparus aurata* using a specific antibody (G7) (Sepulcre et al., 2002). Usually, the head-kidney is the source of the acidophilic granulocytes in the fish testis (Zapata et al., 1996). In gilthead seabream, the acidophilic granulocyte increased their number in the testis during a specific phase of the reproductive cycle (post-spawning stage, just after sperm release), being found into close contact with germ cells, especially to type A undifferentiated spermatogonia (Chaves-Pozoet al., 2018; Chaves-Pozo et al., 2003; Chaves-Pozoet al., 2005). Interestingly, these granulocytes do not trigger any inflammatory response in the testis. On the other hand, the gilthead seabream testicular granulocytes accumulate interleukin (IL)-1β (Chaves-Pozoet al., 2018; Chaves-Pozo et al., 2003; Chaves-Pozoet al., 2005), which is a potent growth factor for mammalian spermatogonia and Leydig cells (Pöllänen et al., 1989; Hedger, 1997) rather than being involved in the phagocytosis of degenerative germ cells (Chaves-Pozo et al., 2003). Moreover, it has been shown IL-1β production is regulated by gonadal steroids in gilthead seabream, indicating that fish leukocytes might be sensitive to sex steroid levels (Chaves-Pozoet al., 2018; Chaves-Pozo et al., 2003). Testicular granulocytes also show higher expression of matrix metalloproteinases genes, which indicates a role of these cells in the testicular remodelation at the end of the reproductive cycle, as shown in gilthead seabream (Chaves-Pozo et al., 2008). For Neotropical species, the role of granulocytes in the testis remains unknown for many species. In the Neotropical catfish *Pimelodus maculatus* testis, it has been observed an increased number of acidophilic granulocytes during the regression and regressed phases of

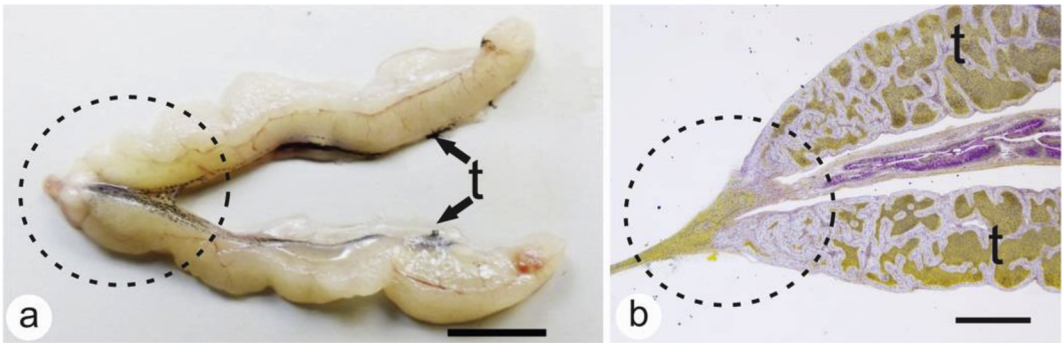

Fig. 1. Testes (t) of *Astyanax altiparanae* in an anatomical (a) and microscopical views (b). In general, testes are paired and elongated organs. Testes are joint in the posterior part to form a short duct, named spermatic duct (dashed line circles). Scale bars: a: 0.5 cm; b: 100 μm. Staining method: b = PAS + Hematoxilin + Metanil yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

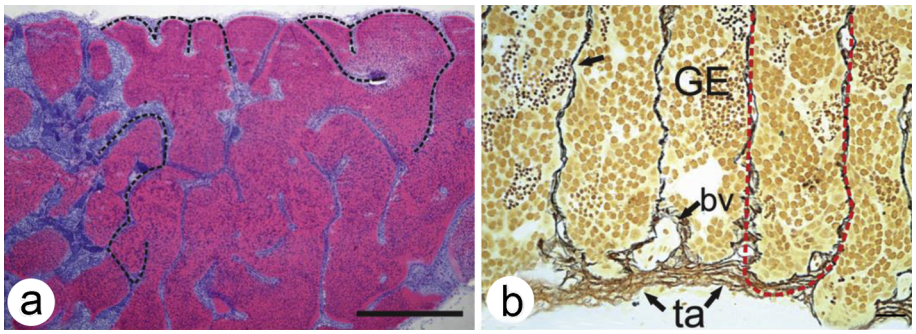

Fig. 2. a) Anastomosing tubular testis of *Astyanax altiparanae*. Dashed lines show the the germinal compartment anastomoses. Note that tubules do not terminate at the testis periphery, but they form loops and anastomoses. (b) Lobular testis of *Cichla kelberi*, evidencing the germinal epithelium (GE) and the interstitium (Arrow). In this case, the germinal compartment ends blindly at the periphery of the testis. Note that tunica albuginea (ta) emits septae (arrow) that divide the testicular parenchyma into the germinal and interstitial compartments. Red dashed line indicates a lobule. Germinal epithelium (GE) and blood vessel (bv) are also indicated. Scale bars: a: 100 μm; b = 10 μm. Staining method: a = Hematoxylin/Eosin; b = Reticulin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the reproductive cycle, where the gonad undergone remodelation and it is mainly composed of spermatogonia and residual sperm (Nóbrega, 2006). Interestingly, the testicular *P. maculatus* granulocytes showed immunoreactivity to membrane type 1 matrix metalloproteinase antibody, which suggests their involvement in the testicular extracellular matrix remodeling at the end of the reproductive cycle (De Oliveira Santana and Quagio-Grassiotto, 2014), similarly to data found in gilthead seabream (Chaves-Pozo et al., 2008). In the Neotropical cichlid *Cichlasoma dimerus*, it has been shown numerous acidophilic granulocytes in the gonadal primordia in the newly-developed male gonad, suggesting a role of granulocytes in the gonadal morphogenesis (Meijide et al., 2005). Considering that granulocytes and other leukocytes may exert several functions in the testis, such as steroidogenesis, gametogenesis and gonadal development, more studies to address their role in Neotropical species are needed.

Based on the germinal epithelium organization, two types of testicular structure are described in fish, the anastomosing tubular and the lobular types, which according to Parenti and Grier (Parenti and Grier, 2004) depend on the phylogenetic position of the concerned species (Fig. 3 and Table 1). In this context, the anastomosing tubular type is found in the most basal fish taxa. In this type, the germinal epithelium is branched and forms anastomoses that do not blind ended at the gonadal periphery, as shown in *A. altiparanae* testis (Fig. 2a). The anastomosing tubular testis is observed in the neotropical catfishes *P. corruscans, Conorhynchos conirostris, P. maculatus, Lophiosilurus alexandri* and *Rhinelepis aspera* (Magno et al., 2011). On the other hand, the most derived species (Neoteleostei) have the lobular testis type structure, in which the germinal epithelium ends blindly at the periphery of the testis, as shown in the Perciformes species *C. dimerus* (Cun et al., 2011) and *C. kelberi* (Siqueira-Silva et al., 2013) (Fig. 2b).

Next to this classification, Grier and colleagues (Grier et al., 1980) observed that type A undifferentiated spermatogonia have a differential distribution along the germinal epithelium in these different types. In the anastomosing tubular testis, the distribution of spermatogonia is unrestricted along the germinal epithelium, as it can be observed in *Leporinus macrocephalus* (Munhoz et al., 2011), *P. maculatus* (De Oliveira Santana and Quagio-Grassiotto, 2014) and *A. altiparanae* (Fig. 4a, b). On contrary, in the lobular testis, spermatogonia can be found restricted to the distal part of the lobule, at the blind end, near to the tunica albuginea, forming an epithelioid tissue (Fig. 5a), as shown in pejerrey *O. bonariensis* (Majhi et al., 2009) (Fig. 5b) and *Hypsolebias sertanejo* (Fig. 5c). In the lobular testis of the non-atherinomorph Neoteleostei, spermatogonia are distributed along the entire length of the germinal epithelium (Fig. 6a), as shown in *S. marmoratus* (43), *C. kelberi* (Siqueira-Silva et al., 2013) and *C. intermedia* (Fig. 6b). This type is classified as unrestricted spermatogonial type. Among the unrestricted type, an intermediate variation of spermatogonial distribution is seen in *C. kelberi* (Siqueira-Silva et al., 2013) and *C. intermedia.* Although distributed along the entire length of the germinal epithelium, type A undifferentiated spermatogonia accumulates at the blind end of the lobules, close to the tunica albuginea (Fig. 7a–c). Recent data has shown that despite of the unrestricted distribution, type A undifferentiated spermatogonia is associated with a microenvironment close to the interstitial compartment, as reported in the anastomosing tubular testes of Nile tilapia (Vilela et al., 2003) and zebrafish (Nóbrega et al., 2010). The preferential location of type A undifferentiated spermatogonia suggests the establishment of a possible spermatogonial stem cell niche in the fish testis (see below) (Nóbrega, 2006; Lacerda et al., 2014).

3. Spermatogonial niche

Little information is available on spermatogonial stem cell niche in fish until now. However, considering the fact that germinal epithelium is conserved among vertebrates (Grier et al., 2016), information available on mammalian spermatogonial niche could be used as reference for studies in fish. Therefore, as defined by De Rooij (De Rooij, 2009), a spermatogonial stem cell niche is considered the area within

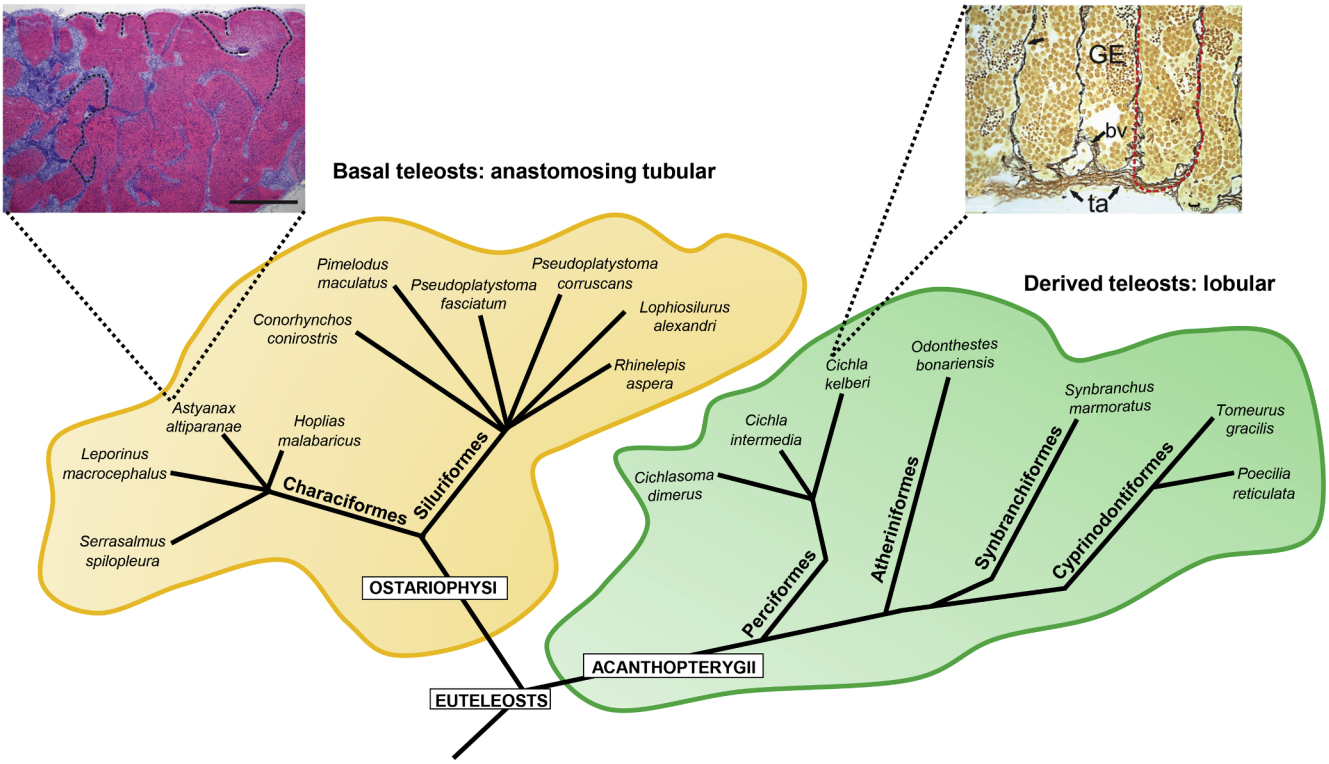

Fig. 3. Phylogenetic diagram illustrating the relation between the testicular structure (anastomosing tubular or lobular) and the phylogenetic position. Anastomosing tubular testis type is typical of the most basal fish, while the most derived species have the lobular testis type structure.

the seminiferous epithelium in which the spermatogonial stem cell can reside and be physically and physiologically maintained throughout life.

In fish, spermatogonial stem cells were detected among type A undifferentiated spermatogonia (Schulz et al., 2010; Nóbrega et al., 2009). In the yellowtail tetra *A. altiparanae*, for example, Rodrigues and colleagues (Rodrigues et al., 2015) described two distinct populations of type A undifferentiated spermatogonia (A_{und*} and A_{und}) by nuclear and cytoplasmic differences, such as shape, size, chromatin condensation and number of nucleoli. These cells exhibited morphological similarity with the type A undifferentiated spermatogonia of zebrafish (Leal et al., 2009). At the moment, the only functional evidence to prove the *stemness* of type A undifferentiated spermatogonia is by germ cell transplantation techniques. Among the transplanted cells, spermatogonial stem cells are the only ones able to self-renewal and differentiate into spermatozoa in the recipient testis (Nóbrega et al., 2010). Still by means of germ cell transplantation assay, spermatogonial stem cells revealed a big plasticity, since they were able to differentiate in female germ cells when transplanted in female hosts (Nóbrega et al., 2010; Takeuchi et al., 2009; Farlora et al., 2014; Yoshizaki et al., 2011).

More recently, special attention has been given to the analyses of the somatic environment that surrounds the spermatogonial stem cell in fish testes, mainly after observation that type A undifferentiated spermatogonia prefer to reside in a microenvironment close to the interstitial compartment (Nóbrega et al., 2010). Nóbrega and colleagues (Nóbrega et al., 2010) analyzed the topographical distribution of type A undifferentiated spermatogonia in zebrafish, and found that 75% of them were located in “niches” near to the interstitium close to Leydig cells and blood vessels (Fig. 8a). Analyses of spermatogonial topographic distribution in the testes of *A. altiparanae* revealed similar

Table 1
Comparison of the testicular structure and spermatogonial distribution in South American Neotropical species.

Neotropical fish species	Distribution of Aund in the germinal epithelium	Testis type structure	References
Astyanax altiparanae	unrestricted	Anastomosing tubular	Costa et al., 2014
Pseudoplatystoma fasciatum	unrestricted	Anastomosing tubular	Batlouni et al., 2006
Pimelodus maculatus	unrestricted	Anastomosing tubular	Magno et al., 2011
Pseudoplatystoma corruscans	unrestricted	Anastomosing tubular	Magno et al., 2011
Lophiosilurus alexandri	unrestricted	Anastomosing tubular	Magno et al., 2011
Rhinelepis aspera	unrestricted	Anastomosing tubular	Magno et al., 2011
Conorhynchos conirostris	unrestricted	Anastomosing tubular	Magno et al., 2011
Leporinus macrocephalus	unrestricted	Anastomosing tubular	Munhoz et al., 2011
Poecilia reticulata	unrestricted	Anastomosing tubular	Billard, 1969
Serrasalmus spilopleura	unrestricted	Anastomosing tubular	Nóbrega, 2003
Cichlasoma dimerus	unrestricted	Lobular	Cun et al., 2011
Cichla intermedia	unrestricted	Lobular	Nóbrega, 2003
Cichla kelberi	intermediate	Lobular	Siqueira-Silva et al., 2015
Odonthestes bonariensis	restricted	Lobular	Majhi et al., 2009
Synbranchus marmoratus	unrestricted	Lobular	Lo Nostro et al., 2003
Tomeurus gracilis	restricted	Lobular	Parenti et al., 2010
Hoplias malabaricus	unrestricted	Lobular	Bizzotto and Godinho (2007)

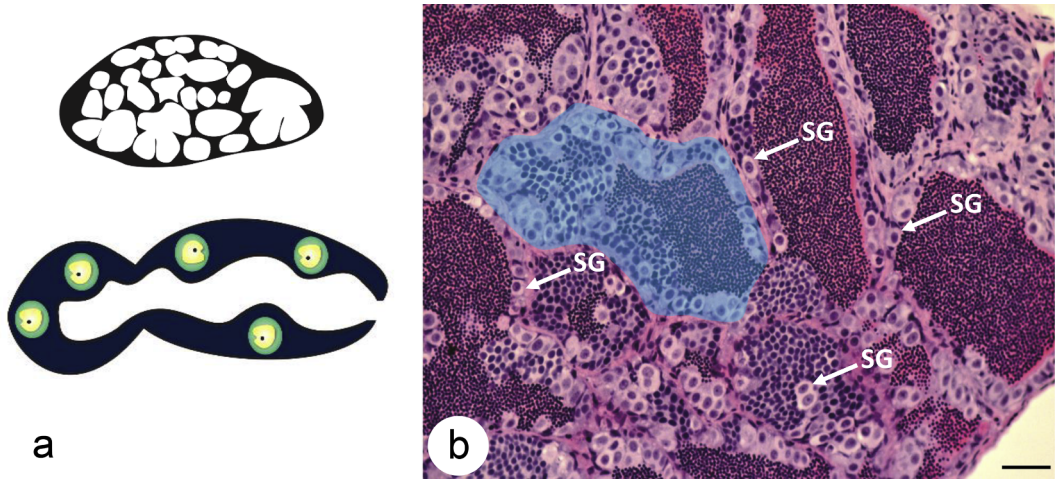

Fig. 4. (a) Diagram illustrating the anastomosing tubular testis (upper part). Note that tubules do not terminate at the testis periphery, but they form loops and anastomoses (lower part). This type is present in most basal fish. (b) *Astyanax altiparanae* testis, highlighting the anastomose of two tubules (blue area), as shown in the diagram (left). Note that spermatogonia (SG) are distributed along the entire length of the germinal epithelium. Scale bar: b: 50 µm. Staining method: Hematoxylin/Eosin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pattern (Rodrigues et al., 2015), the most undifferentiated spermatogonia are located near to the interstitium (Fig. 8b). Likewise, in the species presenting lobular testes (Vilela et al., 2003), type A undifferentiated spermatogonia preferably reside near to the tunica albuginea, as shown in *C. intermedia* (Fig. 7a, b) and *C. kelberi* (Fig. 7c). The tunica albuginea is considered continuous to interstitial compartment, showing interstitial elements, such as Leydig cells and connective tissue (Koulish et al., 2002). Therefore, it seems that interstitial elements (Leydig cells, blood vessels, connective tissue elements among others) seem to be important for the maintenance of type A undifferentiated spermatogonia in the testes of Neotropical teleosts. However, more studies are needed to characterize the spermatogonial niche with respect to its structure and physiological role in the Neotropical species.

4. Sertoli cell structure and function

Sertoli cell is the somatic element of the germinative compartment, and since its first observation by Enrico Sertoli in 1865, this cell has been target of several morphological and physiological studies (França et al., 2016). For many vertebrates, it has been evident that Sertoli-germ cell association is crucial for the germ cell development. Sertoli cell orchestrates and coordinates all phases of spermatogenesis from spermatogonial to spermiogenic phases, ensuring proper spermatogenic development. Moreover, Sertoli cell provides mechanical support for germ cells; testis protection through Sertoli cell barrier; nutritive support for germ cells; phagocytosis of apoptotic germ cells, residual bodies discarded from spermiogenesis and residual sperm; fluid secretion and growth factor production among others (Schulz et al., 2010; França et al., 2016).

Morphologically, Sertoli cells display one of the most interesting

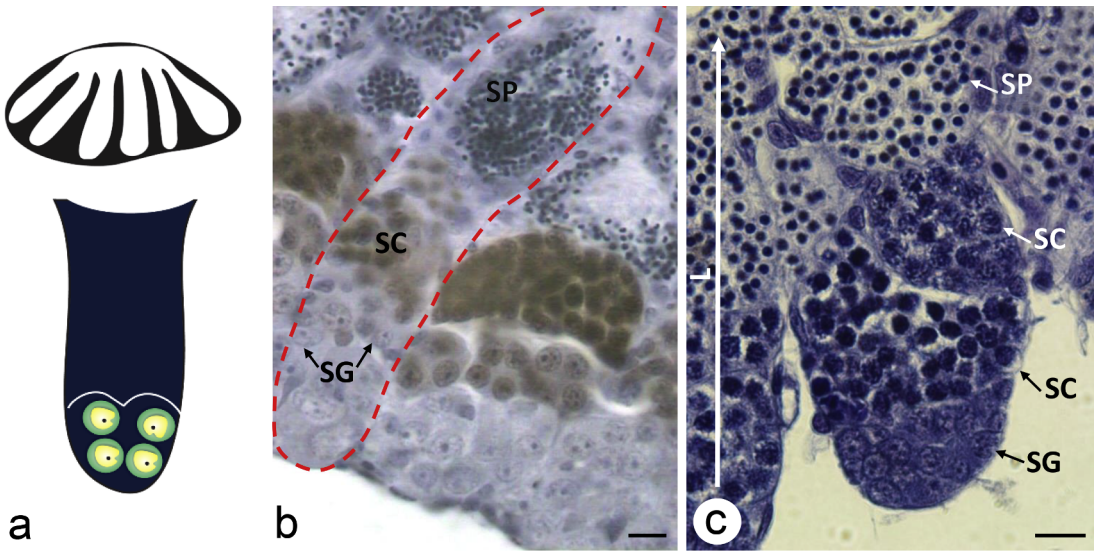

Fig. 5. (a) Diagram illustrating the restricted lobular testis (upper part). In this type, lobules end blindly at the periphery of the testis, near to the tunica albuginea. Note that spermatogonia are restricted to the distal part of the lobule (lower part). This type is found in derived teleost fish. (b) *Odonthestes bonariensis* testis showing a lobule (red dashed line), where spermatogonia (SG) are found at the blind end of the lobule, near to the tunica albuginea. Spermatocytes (SC) and spermatozoa (SP) are also indicated. (c) *Hypsolebias sertanejo* testis. Restricted lobular testis. Spermatogonia (SG), spermatocytes (SC), spermatids (SP), and lobule (L) are shown in the figure. Scale bars: b: 10 µm; c: 20 µm. Staining method: b = PCNA (proliferating cell nuclear antigen) immunodetection; c = Toluidine Blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

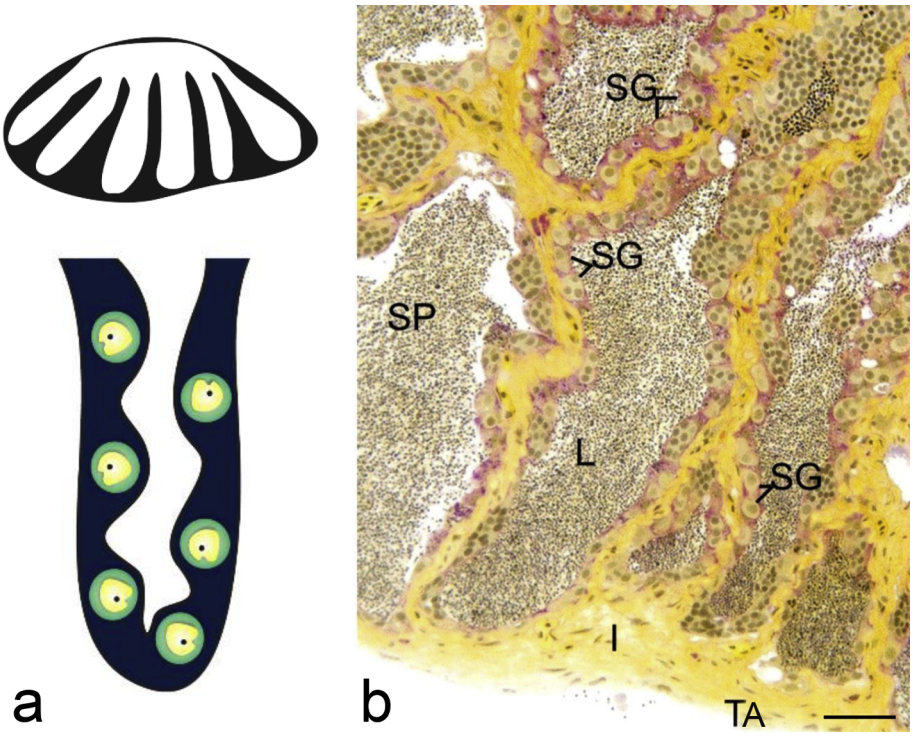

Fig. 6. (a) Diagram illustrating the unrestricted lobular testis (upper part). In this type, lobules end blindly at the periphery of the testis, near to the tunica albuginea. Note that spermatogonia are distributed along the entire length of the germinal epithelium (lower part). (b) *Cichla intermedia* testis showing spermatogonia (SG) spread along the germinal compartment. Interstitium (I), lobule (L), spermatozoa (SP) and tunica albuginea (TA) are indicated. Scale bar: b: 50 µm. Staining method: PAS + Hematoxilin + Metanil yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and complex structures among the existing cells. In fish, Sertoli cells have a large cytoplasmic extension, a very discernible pyramidal and heterochromatic nucleus with a prominent nucleolus that is usually centrally positioned.

Although many features are conserved in vertebrate spermatogenesis, the Sertoli-germ cell relationship differs between anamniote and amniote vertebrates. In amniote (reptiles, birds, mammals) vertebrates, the germinal epithelium in the adult testis is composed of a fixed number of "immortal" Sertoli cells, which support successive waves of spermatogenesis (Sharpe et al., 2003; França et al., 2015). During these waves, one single Sertoli cell supports at the same time different developmental stages of germ cells (i.e. cells belonging to different germ cell clones). For example, the Sertoli cell basis contacts spermatogonia, whereas lateral parts contact spermatocytes and early spermatids, and adluminal parts late spermatids (Sharpe et al., 2003; França et al., 2015). In this type of spermatogenesis, it is interesting that Sertoli cells proliferate until puberty when only spermatogonia and a few early spermatocytes are present in the germinal epithelium (Sharpe et al., 2003; França et al., 2015).

On the other hand, in anamniote vertebrates, cytoplasmic extensions of Sertoli cells form cysts that envelope a single germ cell clone derived from a single stem cell spermatogonium, thus, supporting germ cell at the same stage of development at once (França et al., 2015). The second main difference between amniote and anamniote vertebrates is

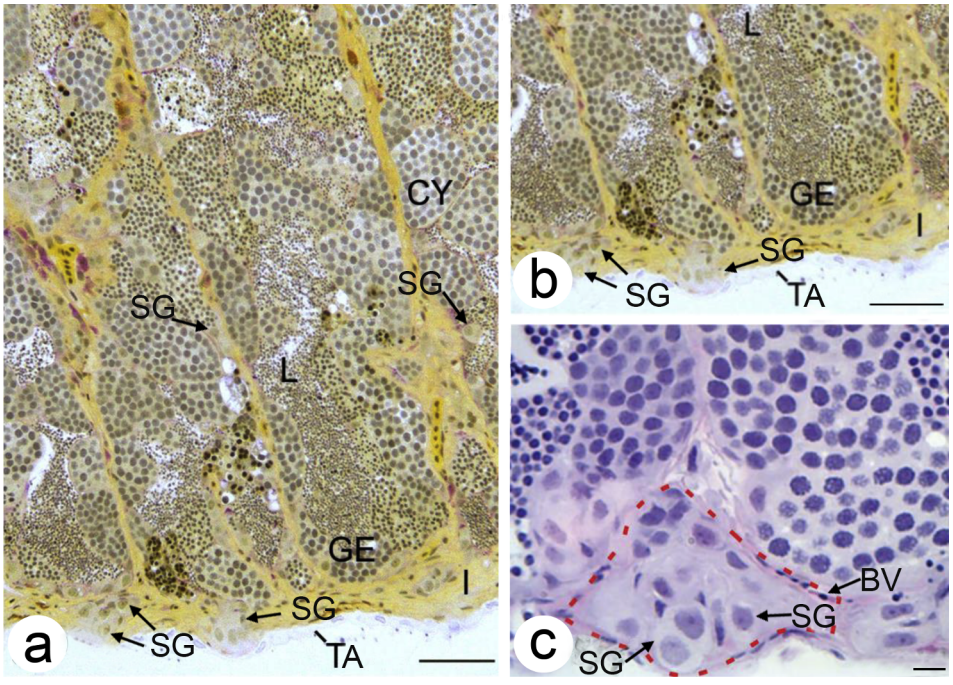

Fig. 7. A variation of the spermatogonial distribution in the unrestricted lobular testis. In this case, spermatogonial cells form clusters at the blind end of the lobule, near to the tunica albuginea. (a,b) *Cichla intermedia* testis showing spermatogonia (SG) spread along the germinal epithelium (GE). Note that spermatogonia form clusters at the blind end of the lobule, near to the tunica albuginea (TA). Interstitium (I), lobule (L) and; spermatocysts or cysts (CY) are shown in the figure. **(c)** *Cichla kelberi* testis showing similar spermatogonial distribution in the germinal compartment. Higher magnification showing a cluster of spermatogonial cells (SG) near to the tunica albuginea. Blood vessel (BV) is indicated. Scale bars: a, b: 50 µm; c: 10 µm. Staining methods: a, b = PAS + Hematoxilin + Metanil yellow; c = Hematoxylin/Eosin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

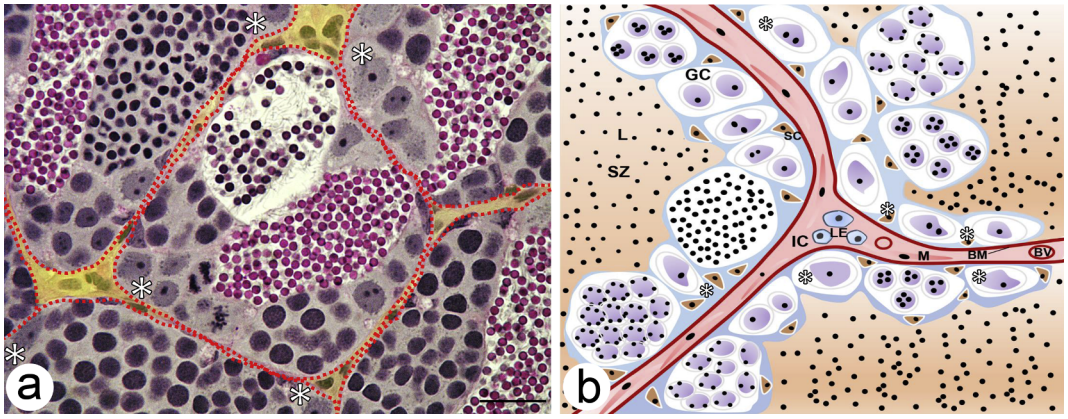

Fig. 8. Spermatogonial niche in teleost fish. (a) Type A undifferentiated spermatogonia (asterisks) are located near to Leydig cells and blood vessels in the interstitial compartment of *Danio rerio* testis. The interstitium is indicated by yellow and surrounded by red dashed lines. (b) Schematical representation of the spermatogonial niche in *Astyanax altiparanae* testis. The asterisks indicate type A undifferentiated spermatogonia, which are located near to the interstitial compartment (IC). Note that type A undifferentiated spermatogonia are distributed close to Leydig cells (LE) and blood vessel (BV). Spermatozoa (SZ), lumen (L), Sertoli cell (SC), peritubular myoid cell (M) and basement membrane (BM) are also indicated in the scheme. Scale bar: a: 10 μm. Staining method: a = PAS + Hematoxilin + Metanil. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

that cyst-forming Sertoli cells retain their capacity to proliferate in adults. In the Neotropical *A. altiparanae,* the number of Sertoli cells per cyst gradually increased from type A undifferentiated spermatogonia to leptotene/zygotene stage of spermatocyte (Rodrigues et al., 2017), similarly as observed in tilapia (Vilela et al., 2003). On the other hand, in *Poecilia reticulata* (Billard, 1969) and zebrafish (Leal et al., 2009), the increase of Sertoli cells was up to spermiogenesis. According to Leal and colleagues (Leal et al., 2009), this is a peculiar feature of the fish spermatogenic development in adults. Moreover, the stabilization of Sertoli cell number at the meiotic cysts can be attributed to the complexity of this phase. At this phase of spermatogenesis, germ cells undergo multiple processes including nucleus division, genetic recombination, and germ cell apoptosis (Schulz and Nóbrega, 2011a,b; Nóbrega et al., 2010).

Interestingly, the increase of Sertoli cell number per cyst dictates the Sertoli-germ cell relationship (Sertoli cell efficiency), which reflects directly the size of the testis, as well as the spermatic production (Sharpe et al., 2003; França et al., 2015; Petersen, 2006). The Sertoli cell efficiency is defined as the ratio between spermatids and Sertoli cells per cyst, in other words, the number of spermatids supported by a single Sertoli cell. In fish, Sertoli cell efficiency is higher than in other vertebrates; humans have the lowest Sertoli cell efficiency (Fig. 9). Studies on Neotropical species, such as *A. altiparanae* (Rodrigues et al., 2017); *Hoplias malabaricus* (Bizzotto and Godinho, 2007); *P. reticulata* (Billard, 1969); *S. spilopleura* (Nóbrega, 2003) and *C. intermedia* (Nóbrega, 2003) showed that a single Sertoli cell supports around 80 to 140 spermatids (Fig. 9). The highest Sertoli cell efficiency was seen in *P. reticulata* (Billard, 1969) and *A. altiparanae* (Rodrigues et al., 2017) (Fig. 9). The elevated Sertoli cell efficiency in fish is attributed to the cystic spermatogenesis, in which Sertoli cell surrounds and nurses a synchronic group of germ cells at the same stage of development at once. This strategy allows an easier and virtuous control of spermatogenesis, increasing the Sertoli cell performance on nursing the germ cells.

Besides their structural function, Sertoli cells can also exert secretory functions in fish testis. In *A. altiparanae*, Sertoli cells form an apocrine secretory epithelium close to the spermatic duct (Costa et al., 2014). The secretory Sertoli cells release a seminal fluid, which can be involved in the spermatozoa nutrition in the testicular lumen (Costa et al., 2014). The Sertoli cells in this region are modified to a columnar shape, showing a cytoplasm full of vesicles and the apical surface usually presenting apocrine secretion. Costa and colleagues (Costa et al., 2014) showed many tight junctions and desmosomes joining the secretory Sertoli cells.

A remarkable characteristic of the fish Sertoli cells is their efficient phagocytic capability, which is the normal physiological function of Sertoli cells (França et al., 2015). Electron microscopy studies have demonstrated Sertoli cell phagocytic activity and shown phagosomes, vesicles with hydrolytic enzymes, lysosomes with degenerating spermatids and spermatozoa, and residual bodies in *S. spilopleura* (Nóbrega and Quagio-Grassiotto, 2007); *S. marmoratus* (Lo Nostro et al., 2003) and *B. affinis* (Andrade et al., 2001). The acid phosphatase detection in Sertoli cell lysosomes is a structural marker of the phagocytic activity of this cell, as reported in *S. spilopleura* (Nóbrega and Quagio-Grassiotto, 2007), *O. niloticus* and *Odonthestes perugiae* (Porawski et al., 2004). Interestingly, flow cytometry studies in gilthead seabream were able to quantify phagocytic activity in the testis and showed that Sertoli cells are the main phagocytic cells in the testis (Chaves-Pozoet al., 2004). Moreover, Sertoli cells of gilthead seabream express macrophages markers, such as macrophage-colony stimulating factor receptor (Mcsfr), which was found in Sertoli cells that formed cysts and in Sertoli cells that limited the spermatic duct just after spermiation (Chaves-Pozoet al., 2009).

5. Perspectives on the Neotropical fish spermatogenesis

Considering that Neotropical region is one of the hotspot regions for the discovery of new fish species, several opportunities of studies may arrive in a soon future. One area that certainly will be reason of investigation is the spermatogenesis of new species with emphasis on basic morphological characterization and phylogenetic classification (Parenti and Grier, 2004; Uribe et al., 2014). Other possibilities are the studies for conservation of endangered and valuable species by germ cell transplantation (Nóbrega et al., 2009; Nóbrega et al., 2010; Majhi et al., 2014), which showed prominent progress mainly due to the basic studies of the donor cells and recipient testis (Panda et al., 2011; Siqueira-Silva et al., 2015). On the other hand, for the species, who had their spermatogenesis already characterized, new possibilities of research will expand our knowledge to better understand the physiological/molecular aspects of testis function and spermatogenesis. Special attention has been given to *A. altiparanae*, which has become an interesting and common Neotropical experimental model. Thus, perspectives on the Neotropical fish spermatogenesis tend not only to increase but also provide new advances in the study of fish biology.

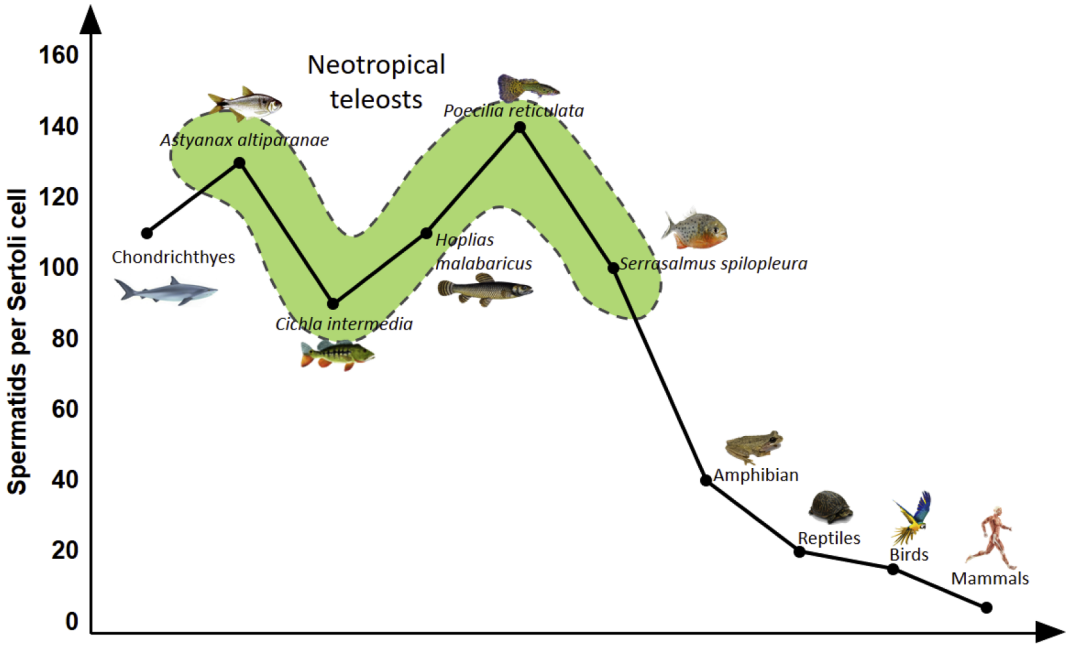

Fig. 9. Sertoli cell efficiency across vertebrates. Sertoli cell efficiency is estimated from the ratio between spermatids and Sertoli cells, i.e. number of spermatids supported by a single Sertoli cell. Neotropical teleost species (*Astyanax altiparanae*, *Cichla intermedia*, *Hoplias malabaricus* and *Poecilia reticulata*) are indicated by green, dashed line area. Note that Sertoli cell efficiency in Neotropical teleost fish is about 10 times higher than most of the mammalian species studied. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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The authors want to dedicate this work to Dr. Harry J Grier (8/7/1940 – 7/4/2018) who devoted his entire life to describe the testicular structure and germinal epithelium in fish. Dr. Grier was a brilliant scientist and his work inspired several South American students and professionals to investigate fish gonadal histology.

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Conflict of interest

The authors declare no conflicts of interest.

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