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Microscopic Evolution of the *Pomadasys jubelini* Testis from the Ivorian Continental Shelf

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Abstract

Undernourishment due to insufficient animal protein is rife in Africa. Fish consumption appears to be the solution to this problem. However, fish products are becoming increasingly scarce due to the depletion of fish farming resources in maritime and continental waters. Hence the need to intensify fish farming. This requires mastery of the reproduction of the fish to be domesticated particularly *Pomadasys jubelini*. Once the fish had been dissected, standard histological techniques were applied to the testicles taken from them. Microscopic study revealed that each testicle lobe is made up of several lobules. Each contained several cysts. Each cyst contained several germ cells of the same generation. Sex cell differentiation proceeded from the periphery to the spermiduct. Stage 4 individuals corresponded to the stage of sexual maturity.

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Keywords

Pomadasys jubelini, testes, germ cells.

Introduction

Undernourishment is a major problem in Africa, especially when it comes to protein intake, and certainly animal protein (Etienne and Felix, 2023).

Fish provides between 20% and 50% of animal protein in Africa (Etienne and Felix, 2023). The fish consumed comes mainly from marine and inland fishing (Weigel, 1998).

Yet 80% of the world's fish stocks are now considered fully exploited or overexploited (Bosanza *et al.,* 2023). Fish farming therefore appears to be the salvation solution for increasing the availability of fish products (Teletchea, 2015). Fish farming requires mastery of the reproduction of the species to be domesticated. Many authors have studied the reproduction of certain species with fish farming potential. Most of the work already done on the reproduction of these fishes has focused on certain parameters such as the size of first sexual maturity, fecundity, oocyte diameter, gonadosomatic ratio and above all the hepatosomatic ratio (Heins *et al.,* 2004; Offem *et al.,* 2009; Sylla *et al.,* 2009; Abba *et al.,* 2012; Tembeni *et al.,* 2014; Bodji, 2015).

The aim of the present work was to study spermatogenesis in *Pomadasys jubelini.*

Materials and Methods

The study involved 92 males of *Pomadasys jubelini*. Sampling was carried out at the fishing port of Abidjan from January 2016 to January 2017. To have intact testes, some fish were dissected on site while others were dissected after a freezing time. All testes were collected for microscopic study.

The reference histological techniques of Martoja and Martoja Pierson (1967) were applied. These involve immersing the testicles in a fixing liquid, then dehydrating them in alcohol. Samples were taken and fixed by immersion in aqueous Bouin liquid or 10% formalin, progressively dehydrated in ethanol (70°, 95° and 100°) and then pre-impregnated in butanol for Bouin-fixed samples and toluene for samples fixed in 10% formalin. Impregnation and embedding were carried out in kerosene (Parafina Para Histologica).

7µm sections were cut with a MICROM microtome and stained with hemalun eosin. The sections were collodionized to prevent detachment. Observation and photos were taken with a Motic and OLYMPUSCKX41 photomicroscope.

Results and Discussion

Stage 1

A transverse section of the testicle (Figure 1 A) showed the presence of a gutter in its dorsal part. The ventral part protruded. A connective wall, the albuginea, enveloped the testicular lobe, which was subdivided into peripheral, intermediate and central zones. The lobe branched into several lobules that converged towards the spermiduct (Figure 1 B). Lobules in the peripheral and intermediate zones contained cysts with spermatogonia (Figure 2 A, B, C and D). The medullary zone contained spermatocytes I (Figure 2 E and F). Spermatogonia ranged in size from 16 µm to 18 µm, while I spermatocytes were between 12 µm and 14 µm.

Stage 2

A cross-section of a testicular lobe (Figure 3 A) showed numerous lobules (Figure 3 B). Spermatocytes I were observed in the cortical and intermediate zones of the lobe (Figure 4 A, B, C and D). The central zone contained spermatocytes II ranging in size from 9 µm to 11 µm (Figure 4 E and F) in the medullary part of the testis.

Stage 3

A transverse section of the testis (Figure 5 A) revealed large cysts. As the cystic membranes thinned and disappeared, contiguous cysts merged to form more elongated cysts (Figure 5 B). Most cysts contained spermatids ranging in size from 5 µm to 7 µm (Figure 6 A, B, C, D, E and F).

Stage 4

A cross-section of a testicular lobe (Figure 7 A) showed large cysts resulting from the coalescence of elongated cysts (Figure 7 B). These various neoformed cysts contained only spermatozoa (Figures 8 A, B, C, D, E and F) between 2 µm and 4 µm in size.

Stage 5

A cross-section of a testicular lobe (Figure 9 A) revealed that the peripheral and intermediate zones were empty (Figures 10 A, B, C and D). The lobules contained residual spermatozoa in the medullary part (Figures 10 E and F). The histological section of a *Pomadasys jubelini* testis shows that it is enveloped in an albuginea. It is subdivided into several lobules of irregular shape and variable size. Each lobule contains several cysts. This observation corroborates the findings of Grier (1993), who showed that the lobular structure of the testes is typical of certain orders of fish, particularly the Perciformes.

Similarly, Otémé (2001); Sylla (2010); Tahari (2011) and Bahou *et al.,* (2016) observed the same lobular structure in *Heterobranchus longifilis*, *Trachinotus teraia*, *Trachurus Trachurus* and *Auxis thazard* respectively. In contrast, Albaret (1976) observed a tubular structure in *Thunnus albacares*. Fantodji (1987) and Bodji (2015) did not mention the lobular organization of testes in *Pomadasys jubelini*, but did note the presence of cysts. Lobule diameter increases from stage 1 to stage 4 in *Pomadasys jubelini*, as the interlobular connective tissue separating two adjacent lobules resorbs and causes them to fuse. This observation was made by Woehl (2001). The testes of *Pomadasys jubelini* were subdivided into three parts: anterior, median and posterior.

The histological section of these different parts shows the same organization. These observations are in line with those of Lehri (1967) in *Clarias batrachus*. On the other hand, Sneed and Clemens (1963) noted that a small part of the posterior region of the catfish testis is glandular and the rest is related to spermatogenic activity.

Figure.1

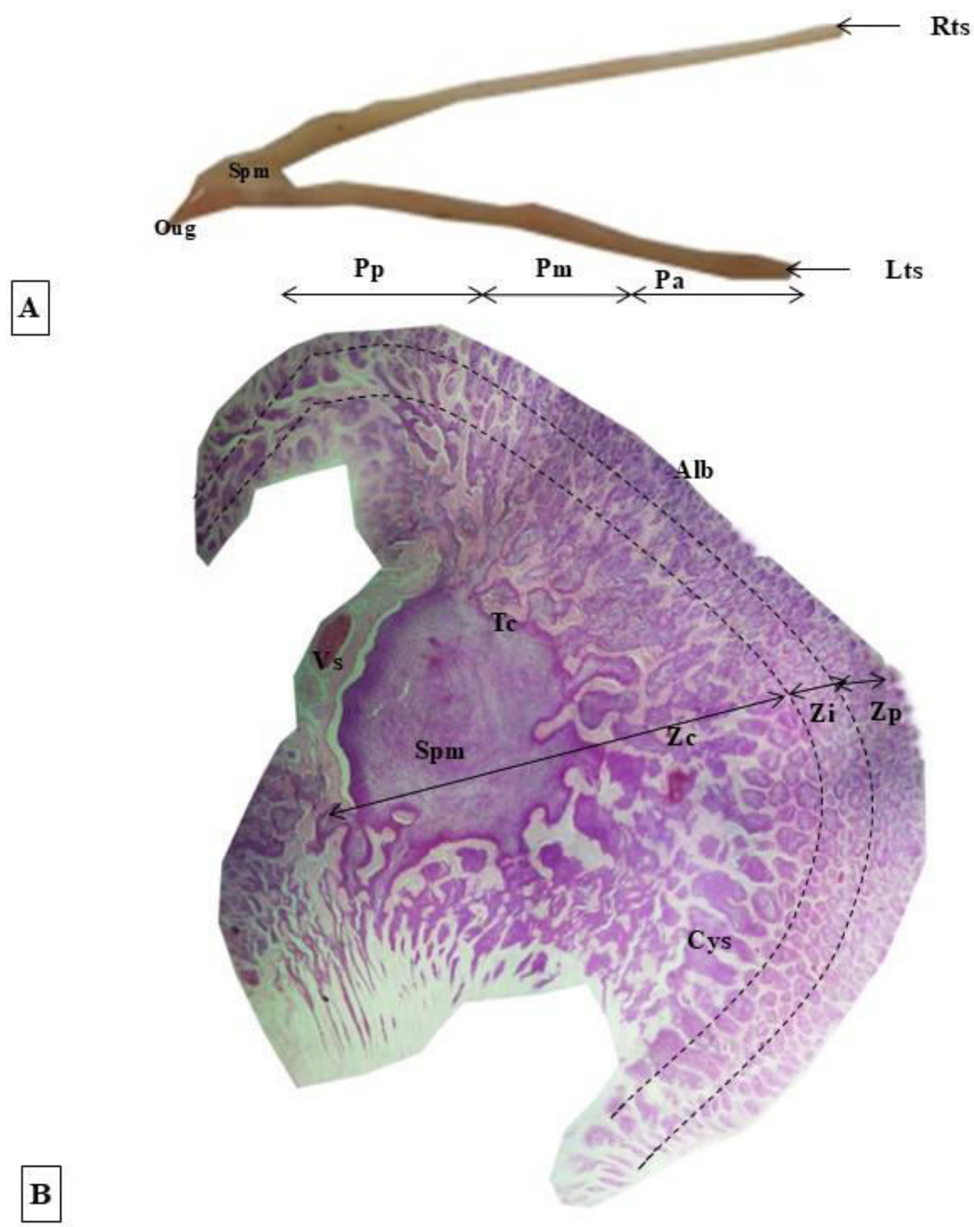

Figure 1: Anatomy and histology of *Pomadasys jubelini* testis at stage 1: A: Anatomy; **B:** overview of a portion of the histological section of the testis; **Zp**: peripheral zone; **Zi**: intermediate zone; **Zc**: central zone; **Rts**: right testis; **Lts**: left testis; **Gt**: gutter; **Spm**: spermiduct; **Pa**: anterior portion; **Pm**: median portion; **Pp**: posterior portion; **Oug**: urogenital orifice; **Tc**: connective tissue; **Cys**: cyst; **Alb**: albuginea, **Bv**: blood vessel
Staining: hemalun-eosin,
Magnification: GX40

Figure.2

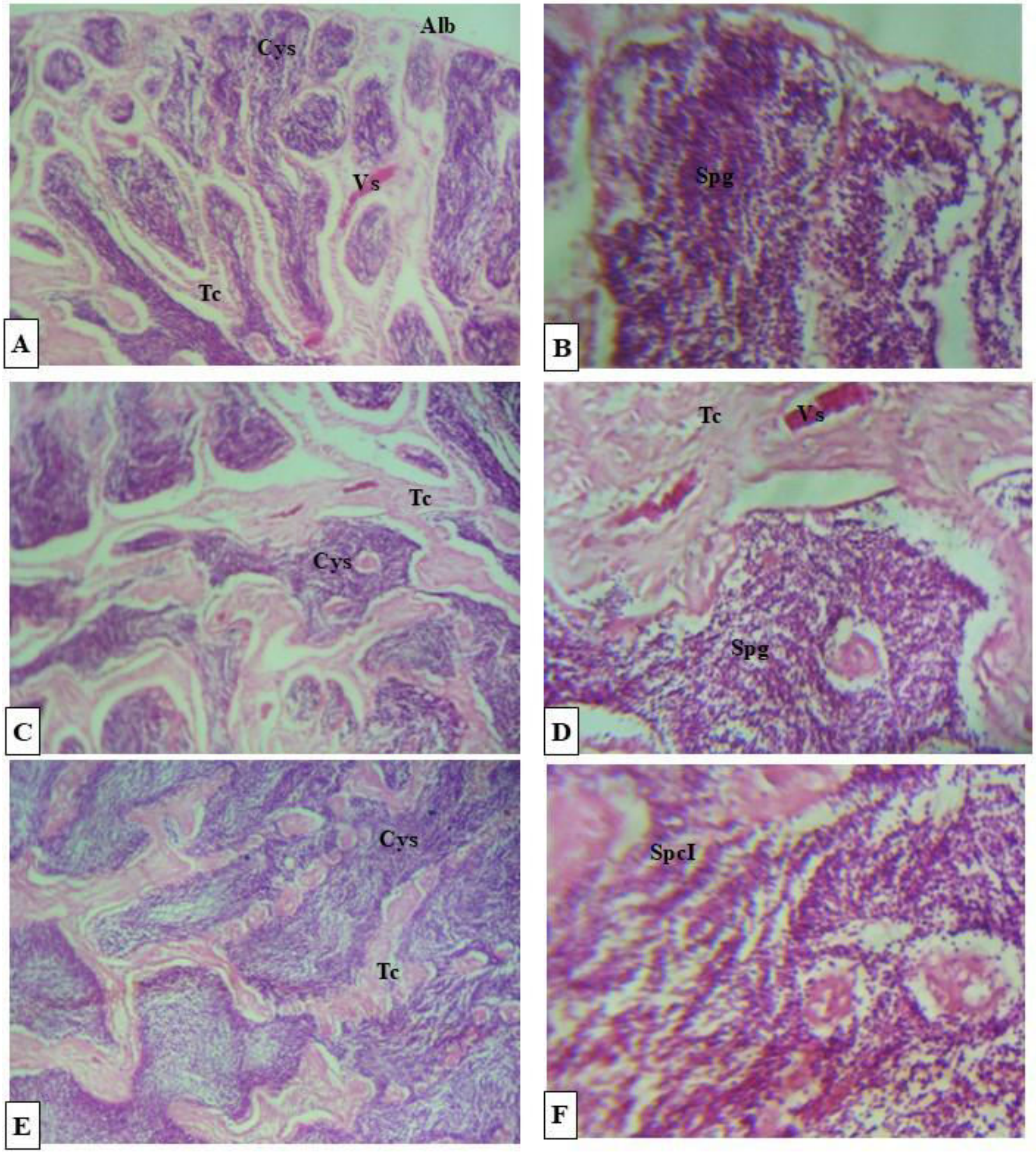

Figure 2: Histology of *Pomadasys jubelini* stage 1 testis: A: view of peripheral zone; **B:** detailed view of cysts in peripheral zone; C: view of intermediate zone; D: detailed view of cysts in intermediate zone; E: view of central zone; F: detailed view of a cyst in central zone; **Tc**: connective tissue**; Cys**: cyst**; Alb**: albuginea**; Spg:** spermatogonia**; Spc I:** spermatocyte **I, Bv:** blood vessel
Staining: hemalun-eosin,
Magnification: A, C and E: G X 100; B; D and F: G X 400

Figure.3

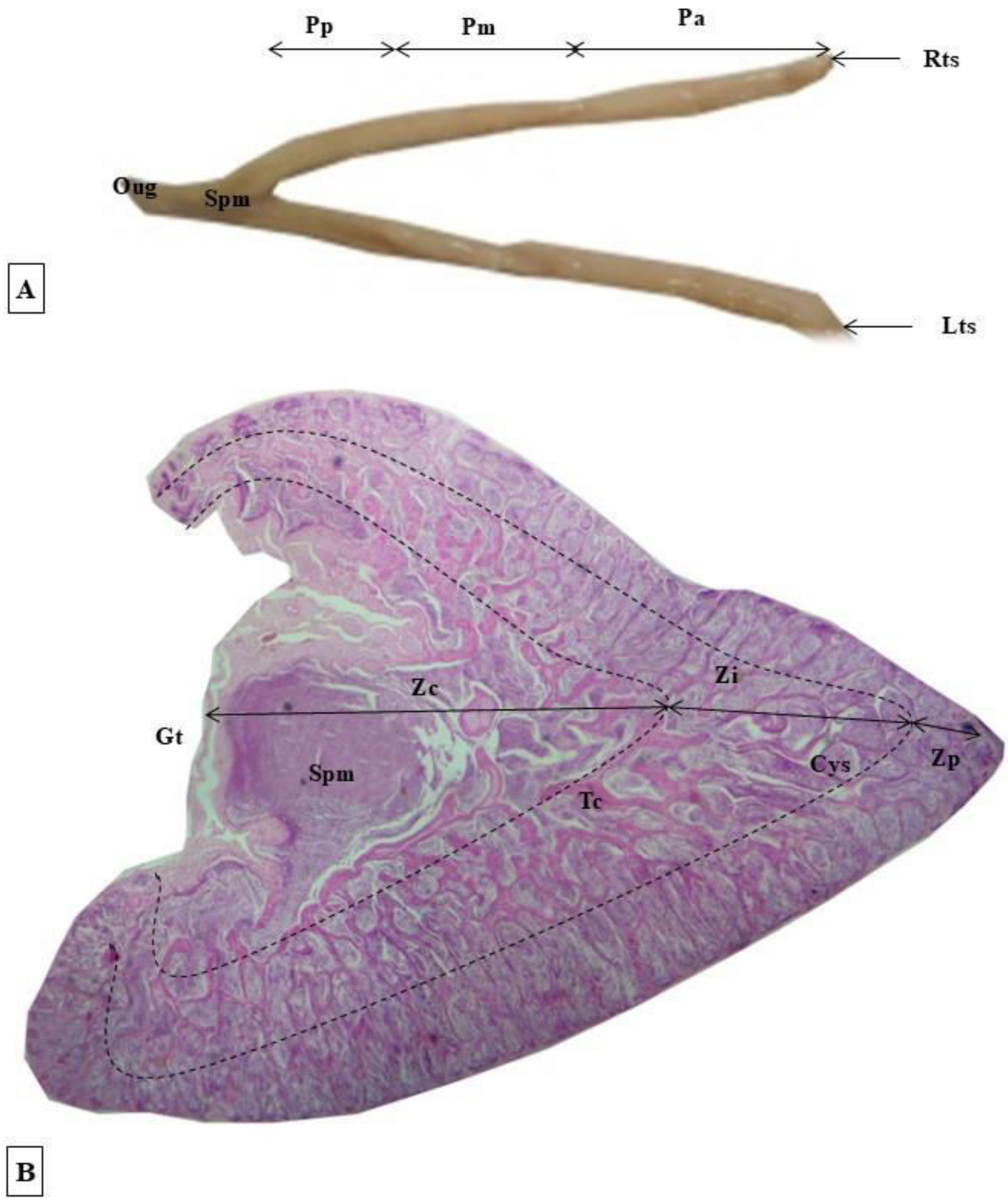

Figure 3: Anatomy and histology of *Pomadasys jubelini* testis at stage 2: A: anatomy; **B:** overview of a portion of the testis; **Zp**: peripheral zone; **Zi**: intermediate zone; **Zc**: central zone; **Rts**: right testis; **Rts**: left testis; **Gt**: gutter; **Spm**: spermiduct; **Tc**: connective tissue; **Cys**: cyst; **Pa**: anterior portion; **Pm**: medial portion; **Pp**: posterior portion; **Oug**: urogenital orifice
Staining: hemalun-eosin,
Magnification: GX40

Figure.4

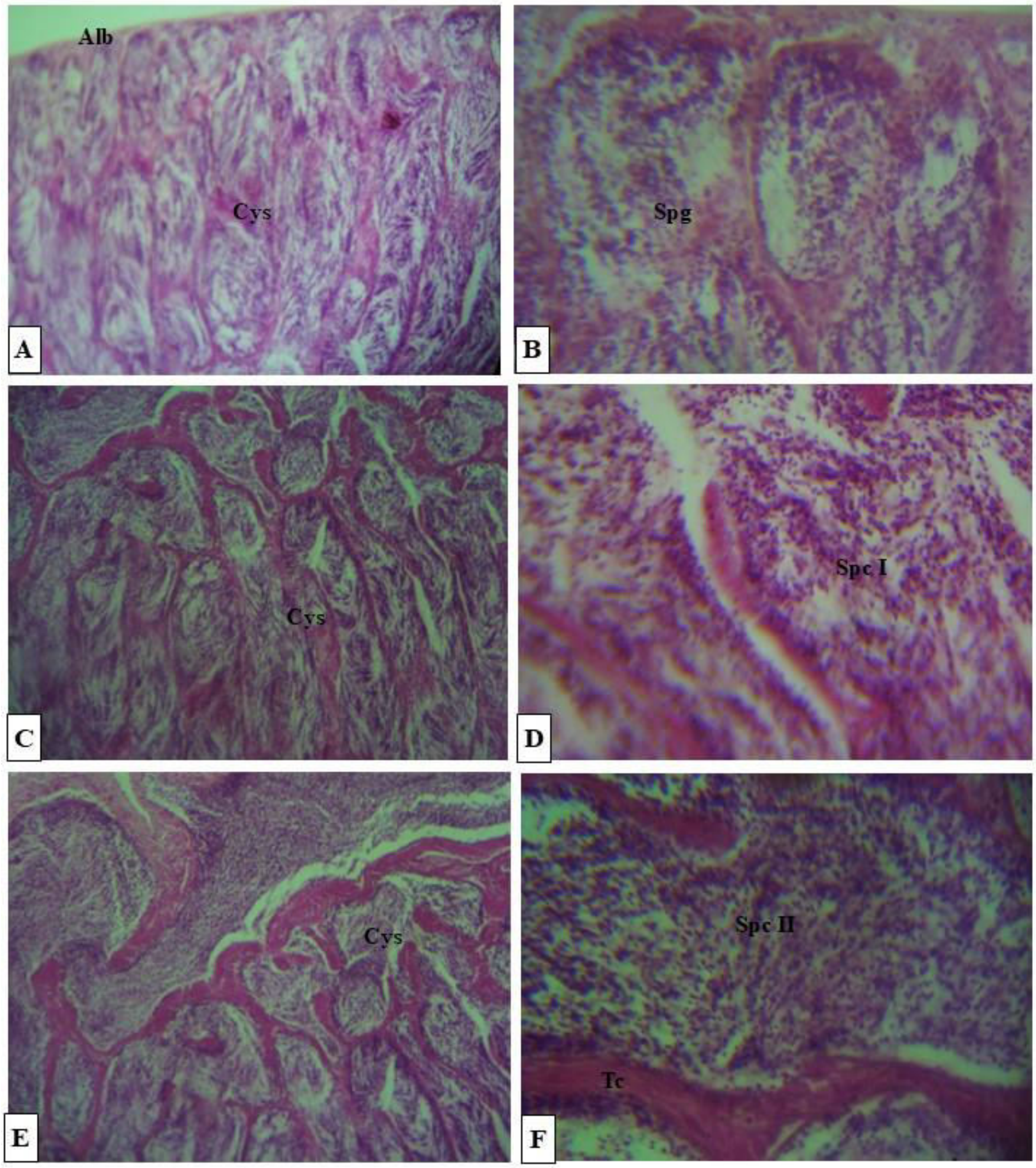

Figure 4: Histology of *Pomadasys jubelini* testis at stage 2: A: view of peripheral zone; **B:** detailed view of cysts in peripheral zone; C: view of intermediate zone; D: detailed view of cysts in intermediate zone; E: view of central zone; F: detailed view of a cyst in central zone; **Tc**: connective tissue; **Cys**: cyst; **Alb:** albuginea; **Spg**: spermatogonia; **Spc I**: spermatocyte **I**; **Spc II**: spermatocyte **II**
Staining: hemalun-eosin,
Magnification: A, C and E: G X 100; B; D and F: G X 400

Figure.5

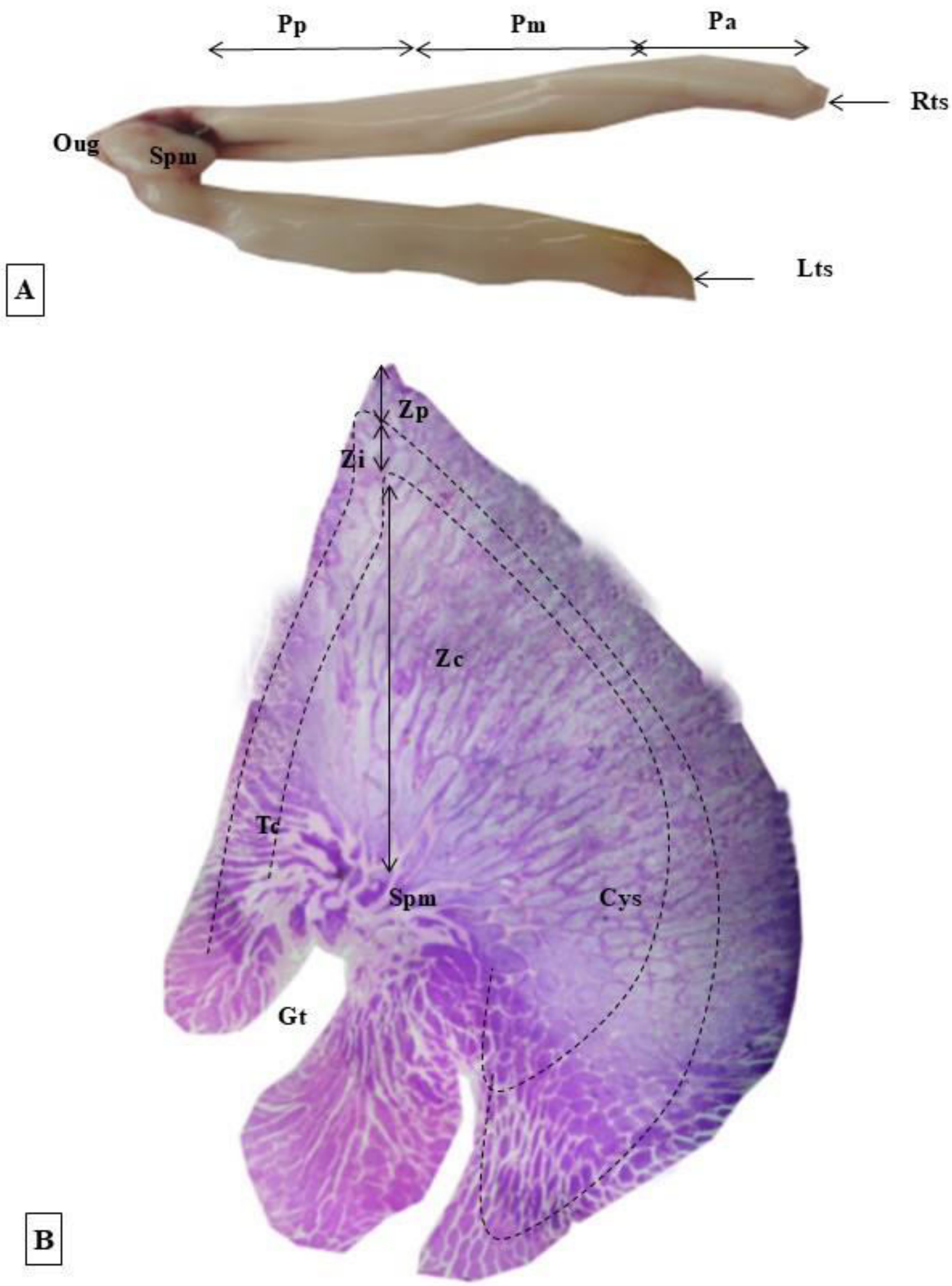

Figure 5: Anatomy and histology of *Pomadasys jubelini* testis at stage 3:
A: anatomy; **B**: overview of a portion of the testis; **Zp**: peripheral zone; **Zi**: intermediate zone; **Zc**: central zone; **Rts**: right testis; **Lts**: left testis; **Gt**: gutter; **Spm**: spermiduct; **Tc**: connective tissue; **Cys**: cyst; **Pa**: anterior portion; **Pm**: medial portion; **Pp**: posterior portion; **Oug**: urogenital orifice
Staining: hemalun-eosin,
Magnification: GX40

Figure.6

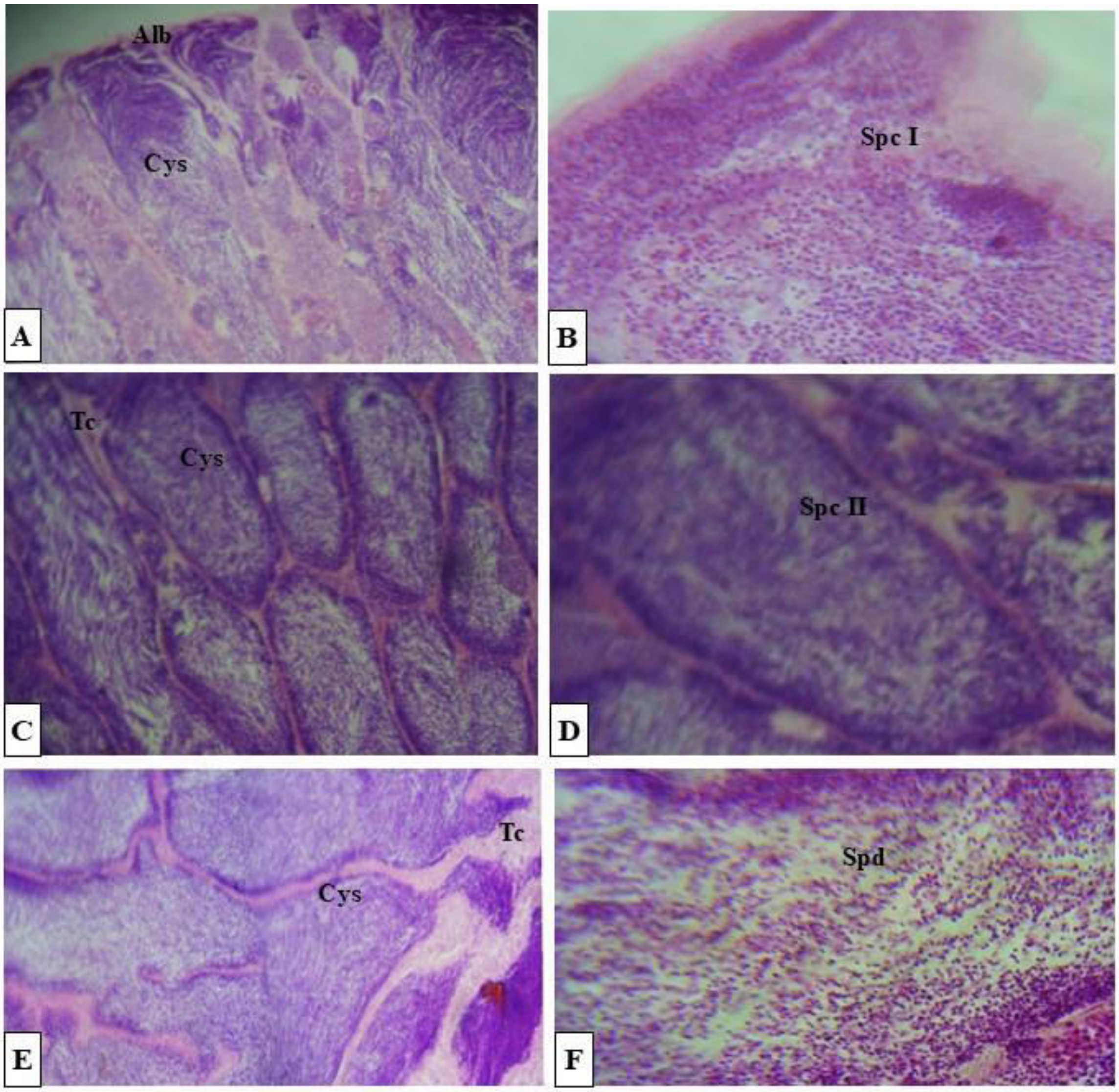

Figure 6: Histology of *Pomadasys jubelini* testis at stage 3: A: view of the peripheral zone; **B:** detailed view of cysts in the peripheral zone; C: view of the intermediate zone; D: detailed view of cysts in the intermediate zone; E: view of the central zone; F: detailed view of a cyst in the central zone; **Tc**: connective tissue**; Cys**: cyst**; Spc I**: spermatocyte I**; Spc II**: spermatocyte II; **Spd**: spermatid.
Staining: hemalun-eosin,
Magnification: A, C and E: G X 100; B; D and F: G X 400

Figure.7

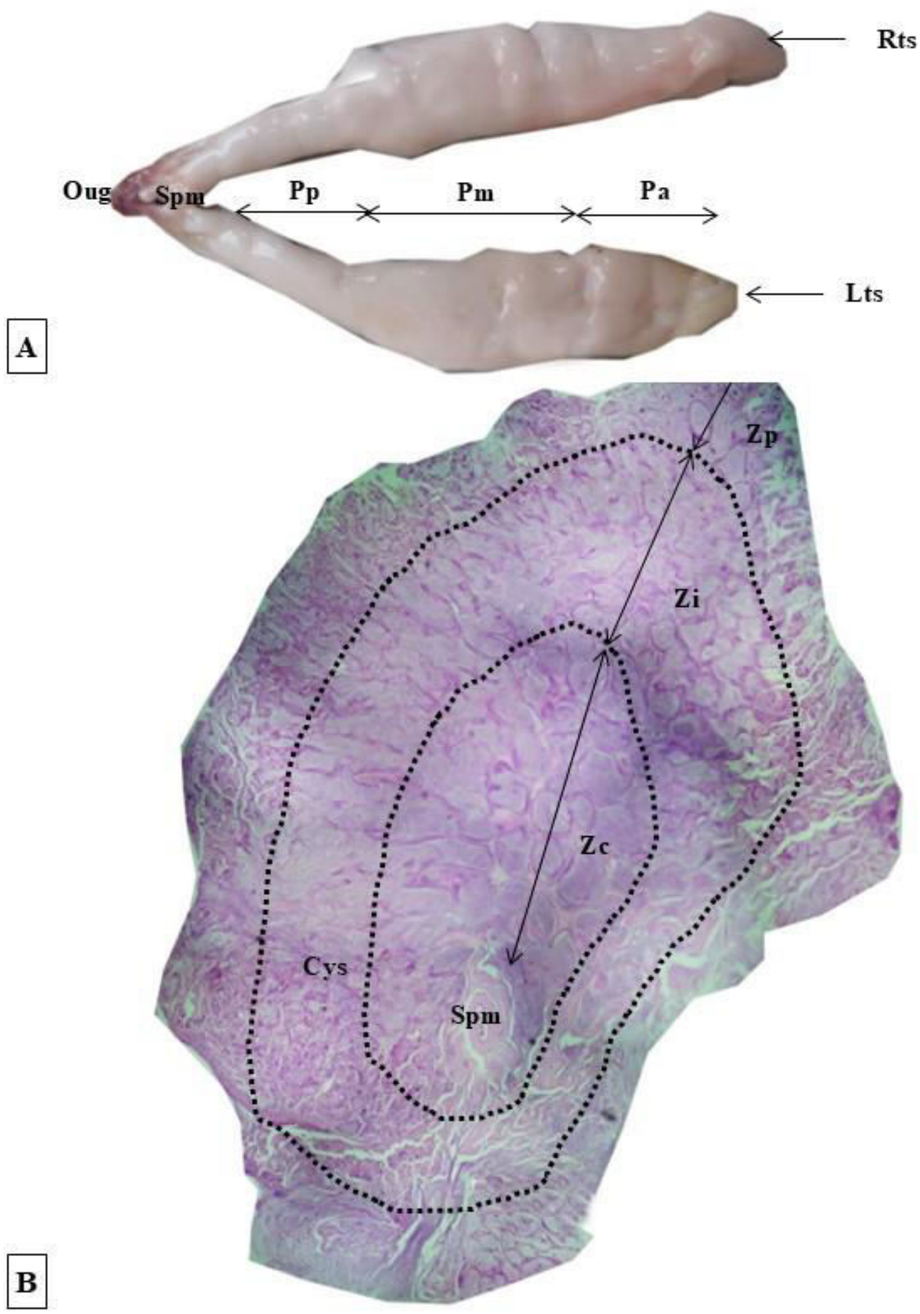

Figure 7: Anatomy and histology of *Pomadasys jubelini* testis at stage 4: A: anatomy; **B:** overview of a portion of the testis; **Zp**: peripheral zone; **Zi**: intermediate zone; **Zc**: central zone, **Rts**: right testis; **Lts**: left testis; **Gt**: gutter; **Spm**: spermiduct; **Tc**: connective tissue; **Cys**: cyst; **Pa**: anterior portion; **Pm**: medial portion; **Pp**: posterior portion; **Oug**: urogenital orifice
Staining: hemalun-eosin,
Magnification: GX40

Figure.8

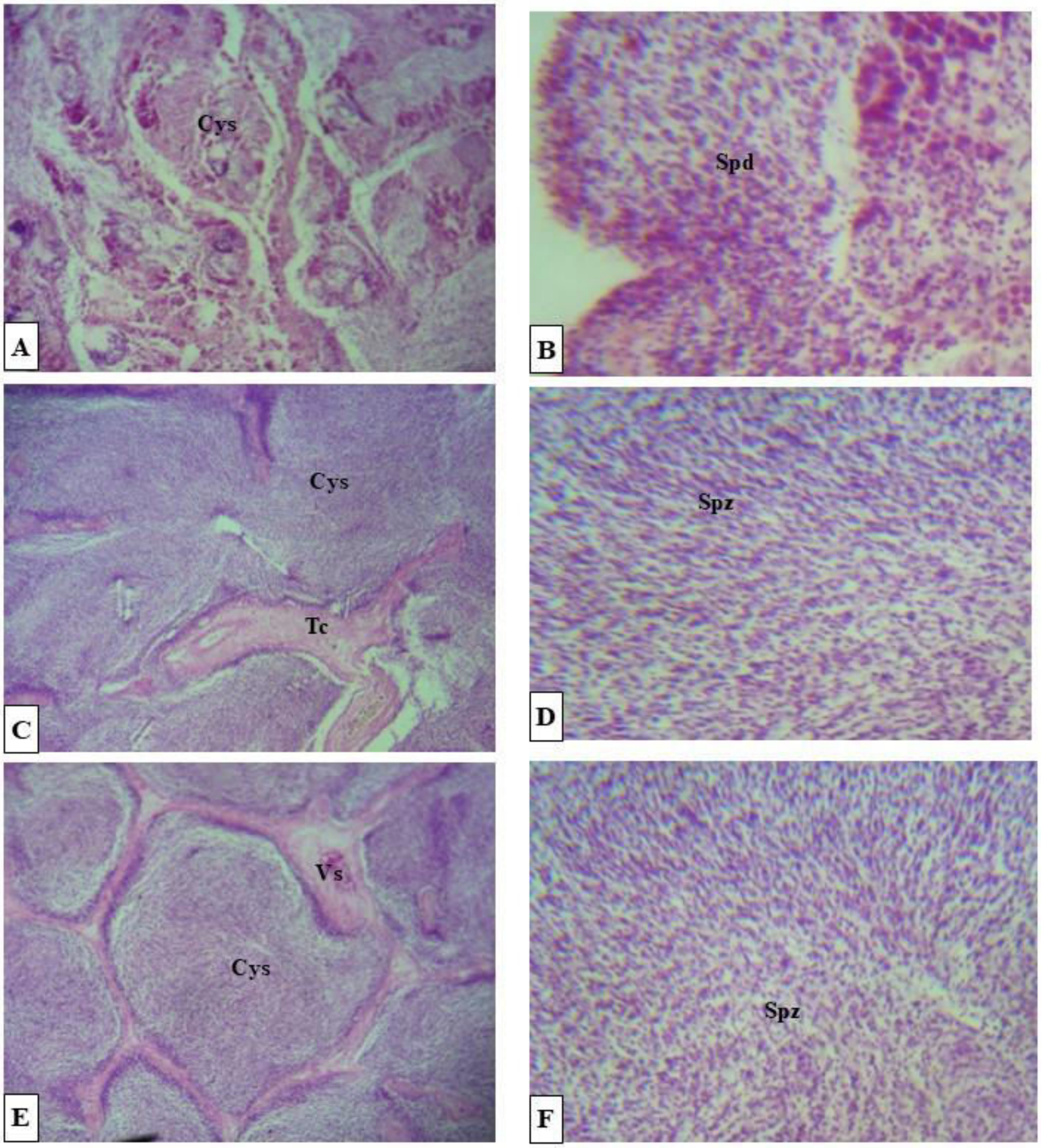

Figure 8: Histology of *Pomadasys jubelini* testis at stage 4: A: view of the peripheral zone; **B:** detailed view of cysts in the peripheral zone; C: view of the intermediate zone; D: detailed view of cysts in the intermediate zone; E: view of the central zone; F: detailed view of a cyst in the central zone; **Tc**: connective tissue**; Cys**: cyst; **Spd**: spermatid; **Spz**: spermatozoon.
Staining: hemalun-eosin,
Magnification: A, C and E: G X 100; B; D and F: G X 400

Figure.9

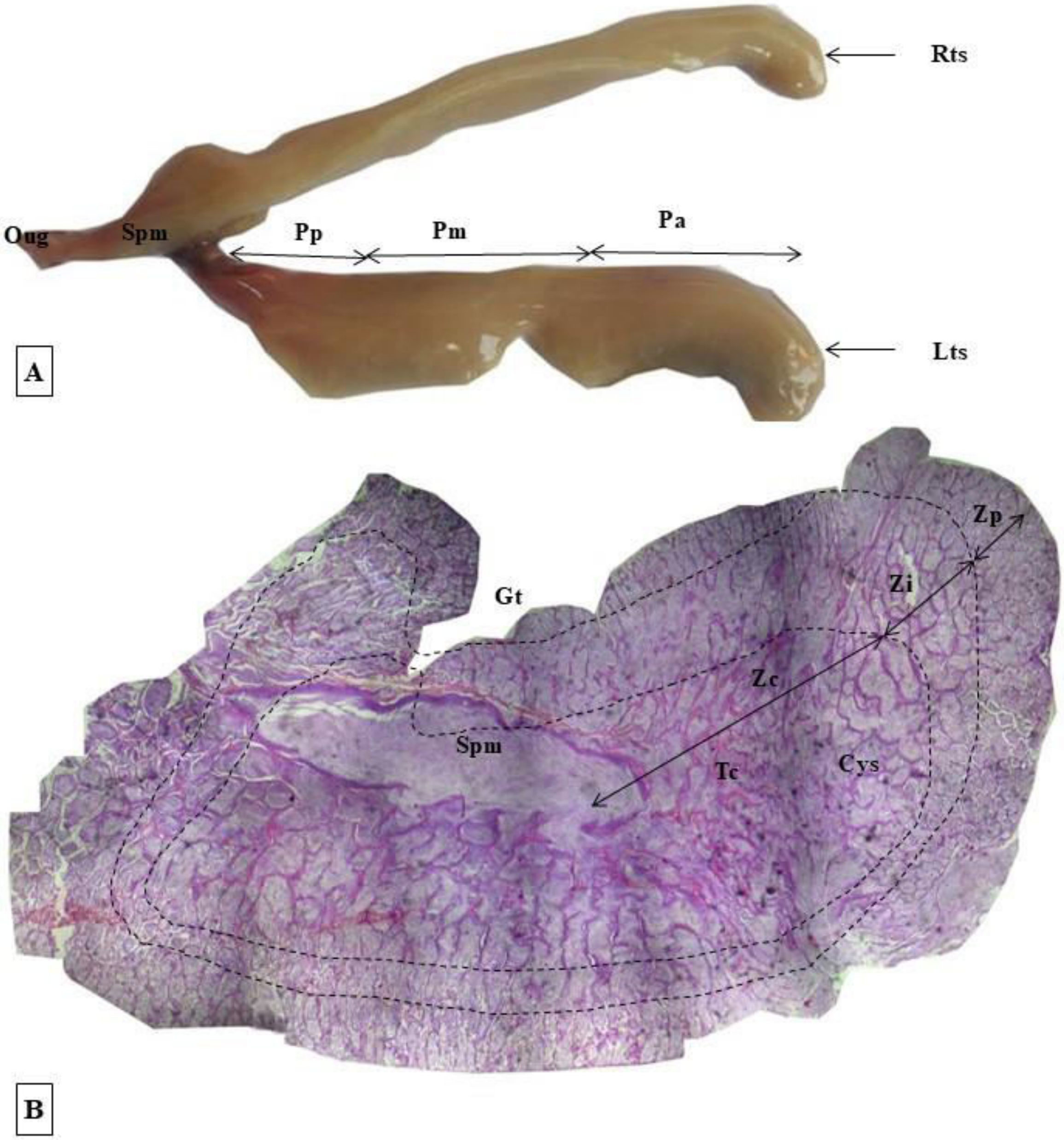

Figure 9: Anatomy and histology of *Pomadasys jubelini* testis at stage 5: A: anatomy; **B:** overview of a portion of the testis; **Zp**: peripheral zone; **Zi**: intermediate zone; **Zc**: central zone; **Tesd**: right testis; **Tesg**: left testis; **Gt**: gutter; **Spm**: spermiduct; **Tc**: connective tissue; **Cys**: cyst; **Pa**: anterior portion; **Pm**: medial portion; **Pp**: posterior portion; **Ug**: urogenital orifice
Staining: hemalun-eosin,
Magnification: GX40

Figure.10

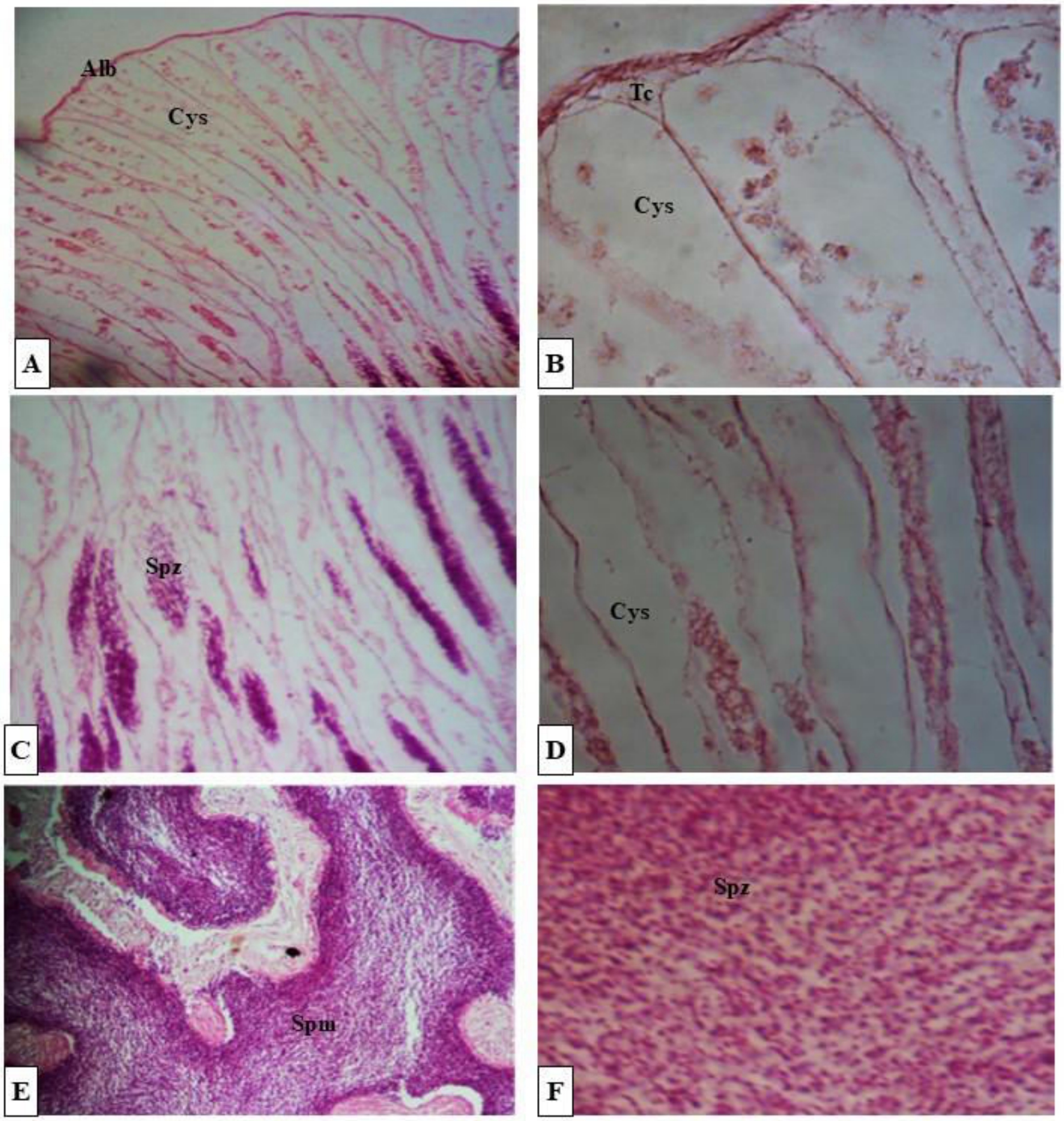

Figure 10: Histology of *Pomadasys jubelini* testis at stage 5: A: view of peripheral zone; **B:** detailed view of cysts in peripheral zone; C: view of intermediate zone; D: detailed view of cysts in intermediate zone; E: view of central zone; F: detailed view of a cyst in central zone; **Tc**: connective tissue; **Cys**: cyst; **Spz**: spermatozoon; **Spm**: spermiduct,
Staining: hemalun-eosin,
Magnification: A, C and E: G X 100; B; D and F: G X 400

Histological sectioning of testes at different stages of maturity *in Pomadasys jubelini* showed a zonation of the testicular structure into three parts, namely the peripheral zone, the intermediate zone and the central zone. Fantodji (1987) and Bodji (2015) did not determine any zonation in *Pomadasys jubelini,* probably because they were unable to observe the lobular structure of the testes. Bahou *et al.,* (2017) observed two zones instead of three, namely the cortical and medullary zones in *Auxis thazard*. In this study, the peripheral and intermediate zones correspond to the cortical zone.

Lobules in the peripheral zone contain less mature sex cells than those in the intermediate and central zone. As a result, sex cells follow a maturation gradient. Maturation is centripetal. These results are in line with those obtained by Bahou *et al.,* (2017), who noted similar germ cell maturation results in *Auxis thazard*. Observation of cells from the same cyst shows that they are all at the same stage of development. These results corroborate those of Fantodji (1987) and Bodji (2015) in *Pomadasys jubelini*.

Spermatogenesis is the process of sperm production. It enables the passage from spermatogonia, diploid cells, to spermatozoa, haploid cells. In male *Pomadasys jubelini*, spermatogenesis takes place during testicular maturation. As pointed out by Schulz *et al.,* (2010), the various stages of spermatogenesis in fish are comparable to those in other vertebrates. In stage 1, only spermatogonia are present in the various zones of the testis. Their number increases as spermatogonia multiply through mitosis. According to Grier *et al.,* (1980), spermatogonia are surrounded by Sertoli cells to form cysts. The spermatogonia produce spermatocytes I. This phenomenon was described by Pudney (1995). At stage 2, spermatocytes I, after the first division of meiosis, transform into spermatocytes II. These results are similar to those of Fantodji (1987), Dziewulska and Domagala (2003) and Bodji (2015). At stage 3, most cysts contain spermatids. These are the products of the second division of meiosis undergone by spermatocytes II (Nagahama, 1983; Dziewulska and Domagala, 2003). At stage 4, haploid spermatids differentiate into spermatozoa without further proliferation. According to Nagahama (1983), spermatids undergo a phase of biochemical and morphological transformations, leading to the formation of spermatozoa. In *Pomadasys jubelini*, from spermatogonia to spermatozoa, germ cells vary in size from 16 to 18 µm, 12 to 14 µm, 8 to 10 µm, 5 to 7 µm and 2 to 4 µm respectively. Germ cell size varies according to species. Under the light microscope, only the nuclei in sperm heads appear ponctiform. During the evolution of germ cells, nuclei undergo mitotic and meiotic divisions. These divisions make the nuclei smaller and smaller. Bodji's (2015) work on *Pomadasys jubelini* showed that spermatogonia are 31.5-38.5 µm in size, spermatocytes I are 15-25 µm, spermatocytes II are 10-12 µm, spermatids are 8-10 µm and spermatozoa are less than 8 µm.

Conclusion

The lobe of the *Pomadasys jubelini* testis is subdivided into several lobules. Each lobule contains cysts. A cyst contains sex cells of the same generation. This histological study of the *Pomadasys jubelini* testis shows that stage 1 and stage 2 individuals are immature. They reach puberty in stage 3, become spermiants in stage 4 and post-spermiants in stage 5. Stage 4 males are therefore ideal broodstock for fish farming.

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