

Supplementary material

Sexual development and maturity of the male brown banded bamboo shark, *Chiloscyllium punctatum* (Elasmobranchii : Hemiscylliidae)

Hesam Sephid^A, *Negin Salamat*^{A,B} and *Mohammad Ali Salari*^A

^ADepartment of Marine Biology, Faculty of Marine Sciences, Khorramshahr University of Marine Science and Technology, Khorramshahr, 64199-34619, Iran.

^BCorresponding author. Email: salamatnegin@gmail.com

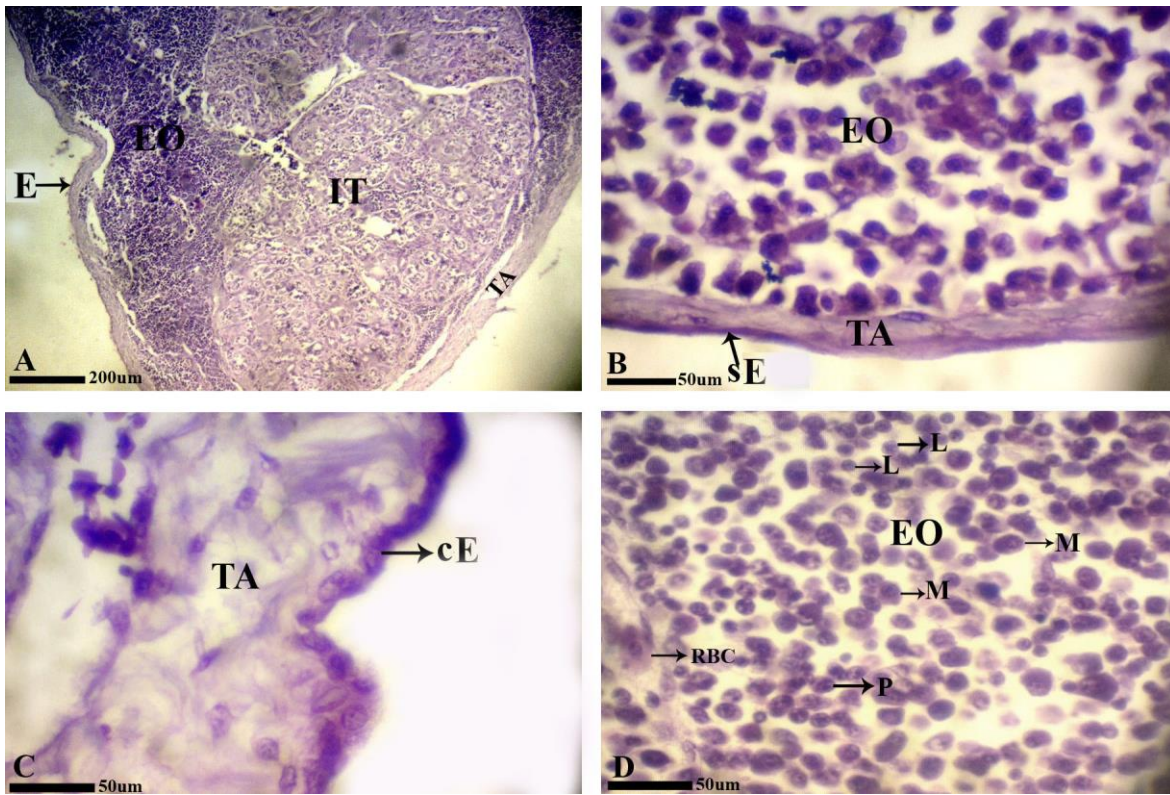


Fig. S1. Representative photomicrograph of testis and epigonal organ in male *Chiloscyllium punctatum*: A. Immature testis (IT), the epigonal organ (EO), the epithelium (E), tunica albuginea (TA); B: Epigonal organ (EO), squamous epithelium (sE), and tunica albuginea (TA) in Immature male; C. Cuboidal epithelium (cE) and tunica albuginea (TA) in mature male; D. Epigonal organ (EO): Macrophages (M), erythrocytes (RBC), lymphocytes (L), plasma cells (P); A (H&E; 290×), B,C,D (H&E; 2900×), E,F (H&E; 7250×).

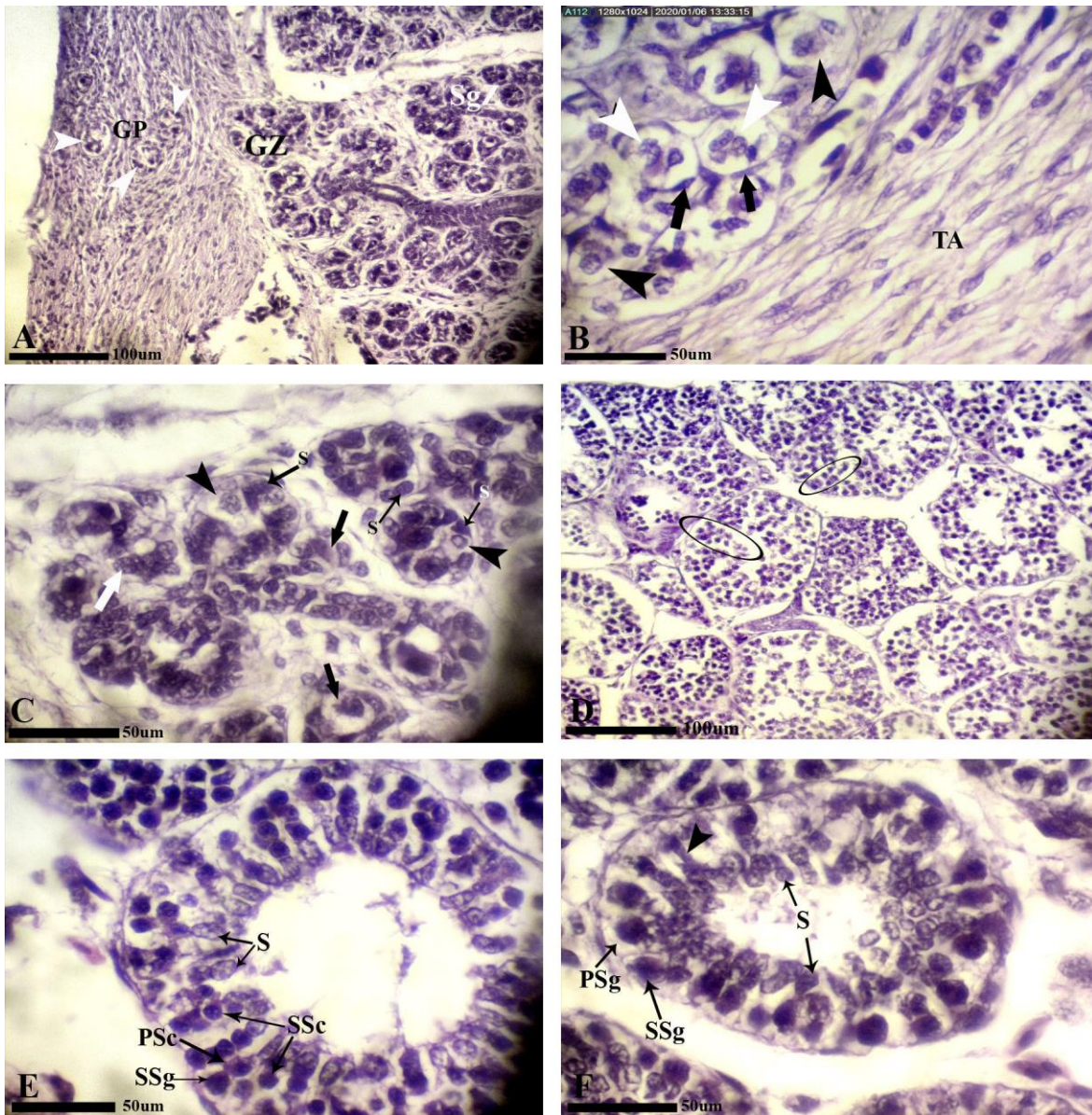


Fig. S2. Representative photomicrograph of different zones in the testicular lobules in male *Chiloscyllium punctatum*; A. Germinal papilla (GP): undifferentiated cells (white arrowheads), germinal zone (GZ), spermatogonial zone (SgZ); B. Germinal zone: single primary spermatogonia (black arrowheads), groups of two to three primary spermatogonia (white arrowheads), progenitor sertoli cells (black arrows), tunica albuginea (TA); C. Spermatogonial zone with one layer spermatocysts: primary spermatogonia with wide vacuolated cytoplasm (black arrowheads), secondary spermatogonia with thin cytoplasm (white arrows), sertoli cells (S), single primary spermatogonia (black arrows); D. Spermatocyte Zone: spermatoblasts (black circles); E,F. Spermatocyte Zone: spermatocysts contained primary (PSg) and secondary (SSg) spermatogonia, primary (PSc) and secondary (SSc) spermatocytes, sertoli cells (S), spermatogonia in cell division (black arrowhead); A,D (H&E; 725×), B,C,E,F (H&E; 2900×).

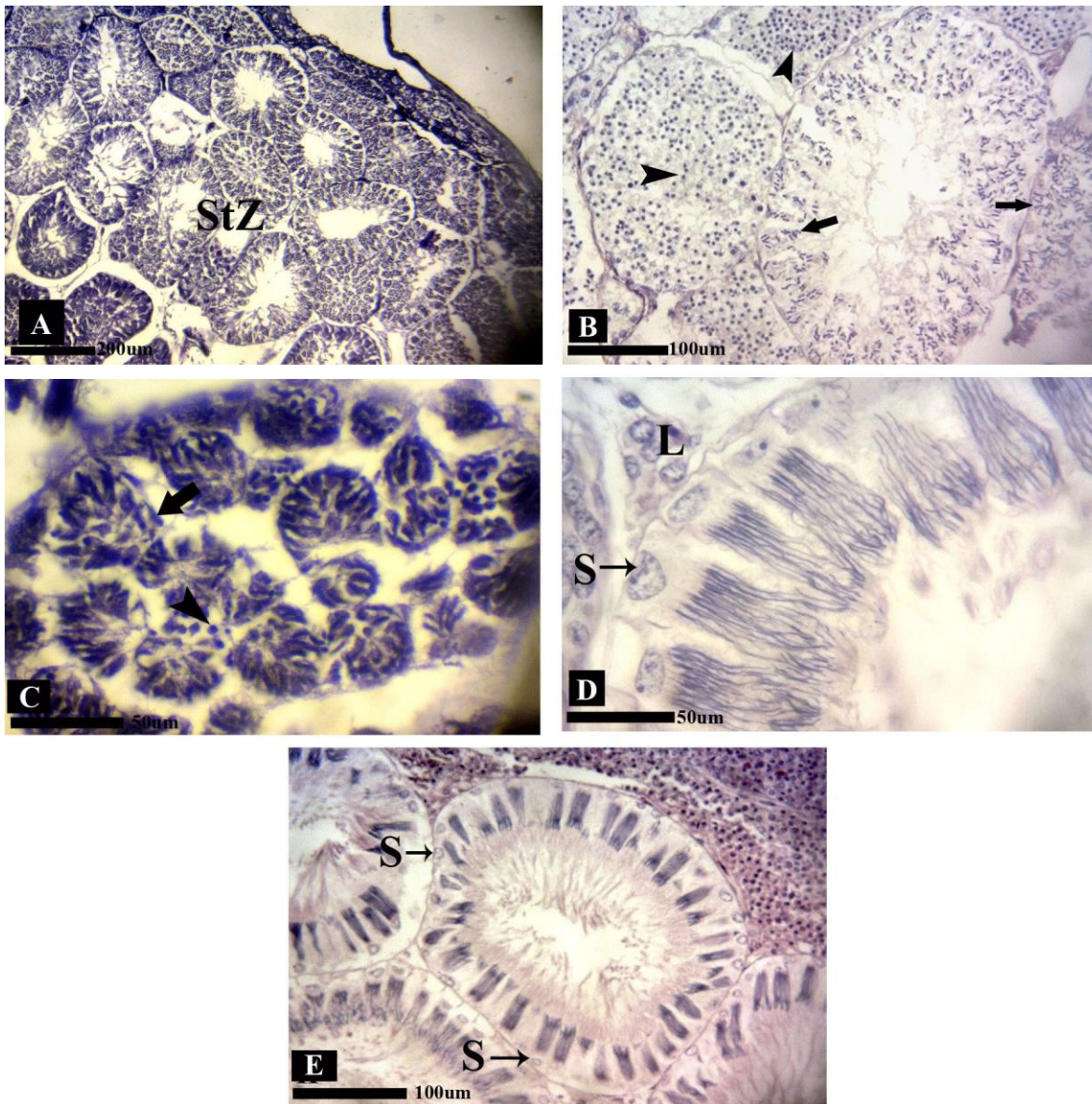


Fig. S3. Representative photomicrograph of spermatid zone in the testicular lobules in male *Chiloscyllium punctatum*; A. Spermatid zone (StZ); B-E. Spermatocysts with spermatids in spermiogenesis: B, C. spermatids with round nucleus (black arrowheads) and rod-shaped nucleus (black arrows), D, E. sertoli cells (S), leydig cells (L); A (H&E; 290×), B,E (H&E; 725), C,D (H&E; 2900×).

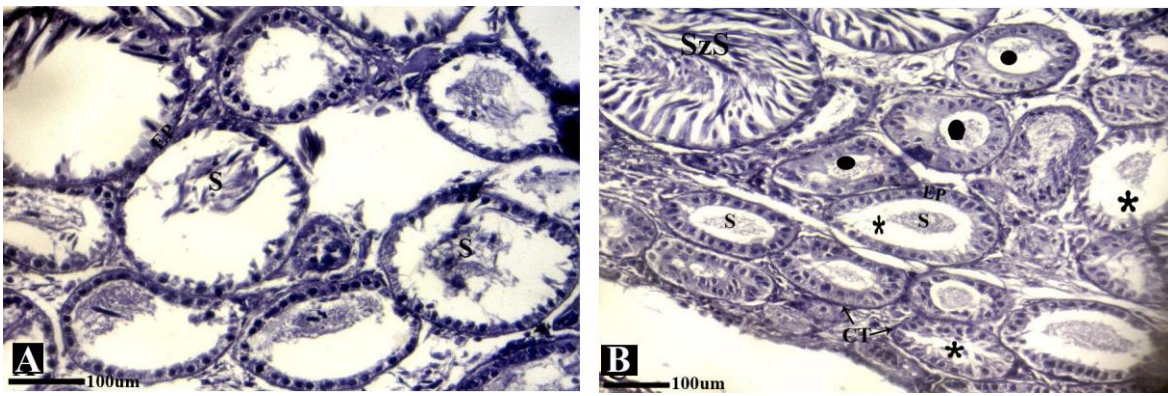


Fig. S4. Representative photomicrograph of the efferent ducts with a thin (A) and thick (B) simple cuboidal epithelium in maturing and adult male *Chiloscyllium punctatum* respectively; Epithelium (EP), Connective tissue (CT), Spermatozoa (S), Spermatozoa spermatocyst (SzS), Efferent ducts with a thick epithelium (black circles) and a thin epithelium (black stars); A,B (H&E; 725×).

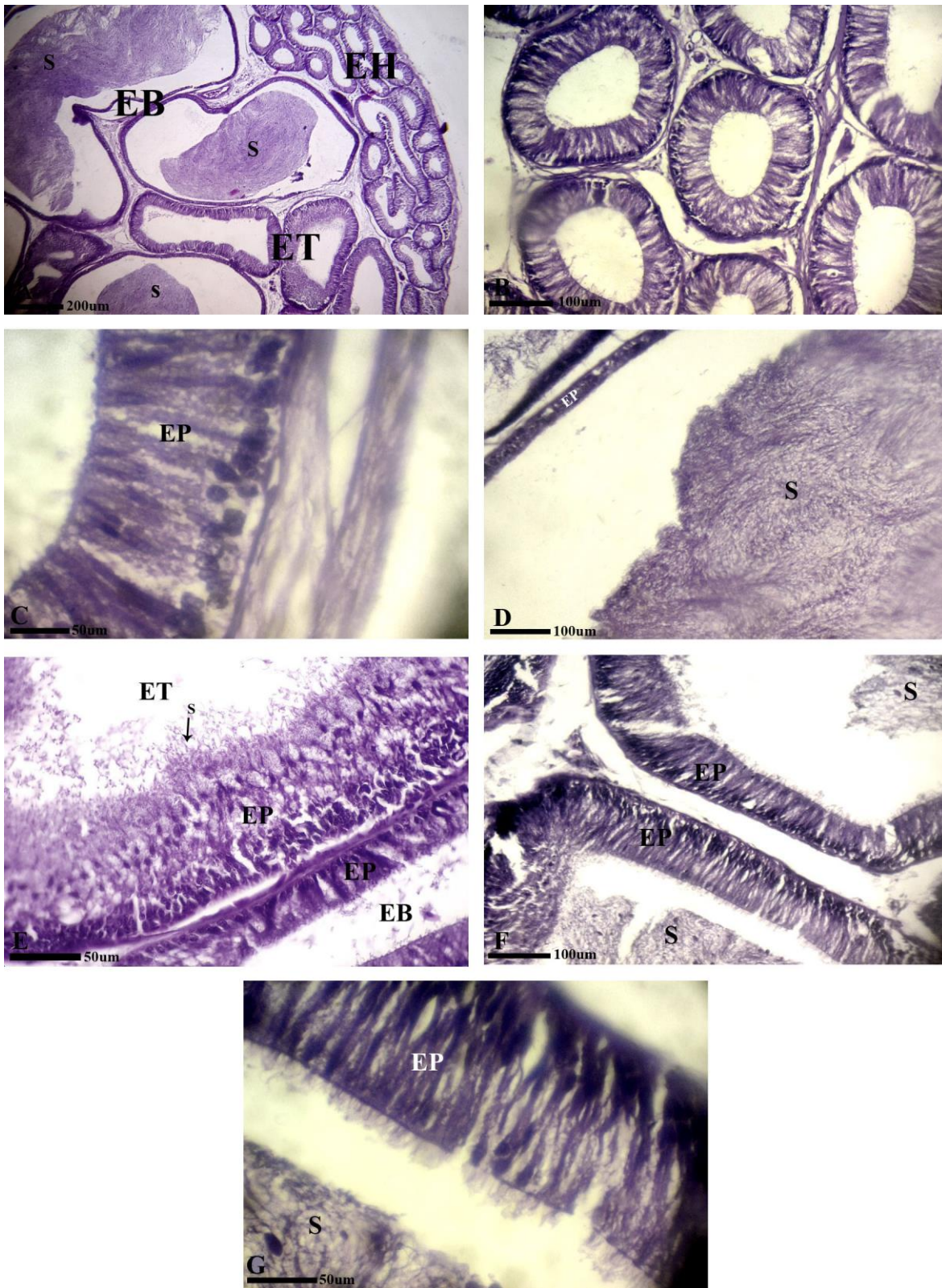


Fig. S5. Representative photomicrograph of the epididymides in active mature male *Chiloscyllium punctatum*; A. Epididymal tubules of the head (EH), body (EB) and tail (ET) regions; B,C. Tubules of the epididymal head lined by a pseudostratified columnar epithelium; there was no spermatozoa in the lumen; D. Tubules of the epididymal body lined by a simple cuboidal epithelium; the lumen was full of spermatozoa; E. Tubules of the epididymal body (EB) and tail (ET); F,G. Tubules of the epididymal tail lined by a pseudostratified columnar epithelium; the volume of spermatozoa reduced in these tubules compared to the body region; Epithelium (EP), Spermatozoa (S); A (H&E; 290 \times), B,D,F (H&E; 725 \times), C,E,G (H&E; 2900 \times).

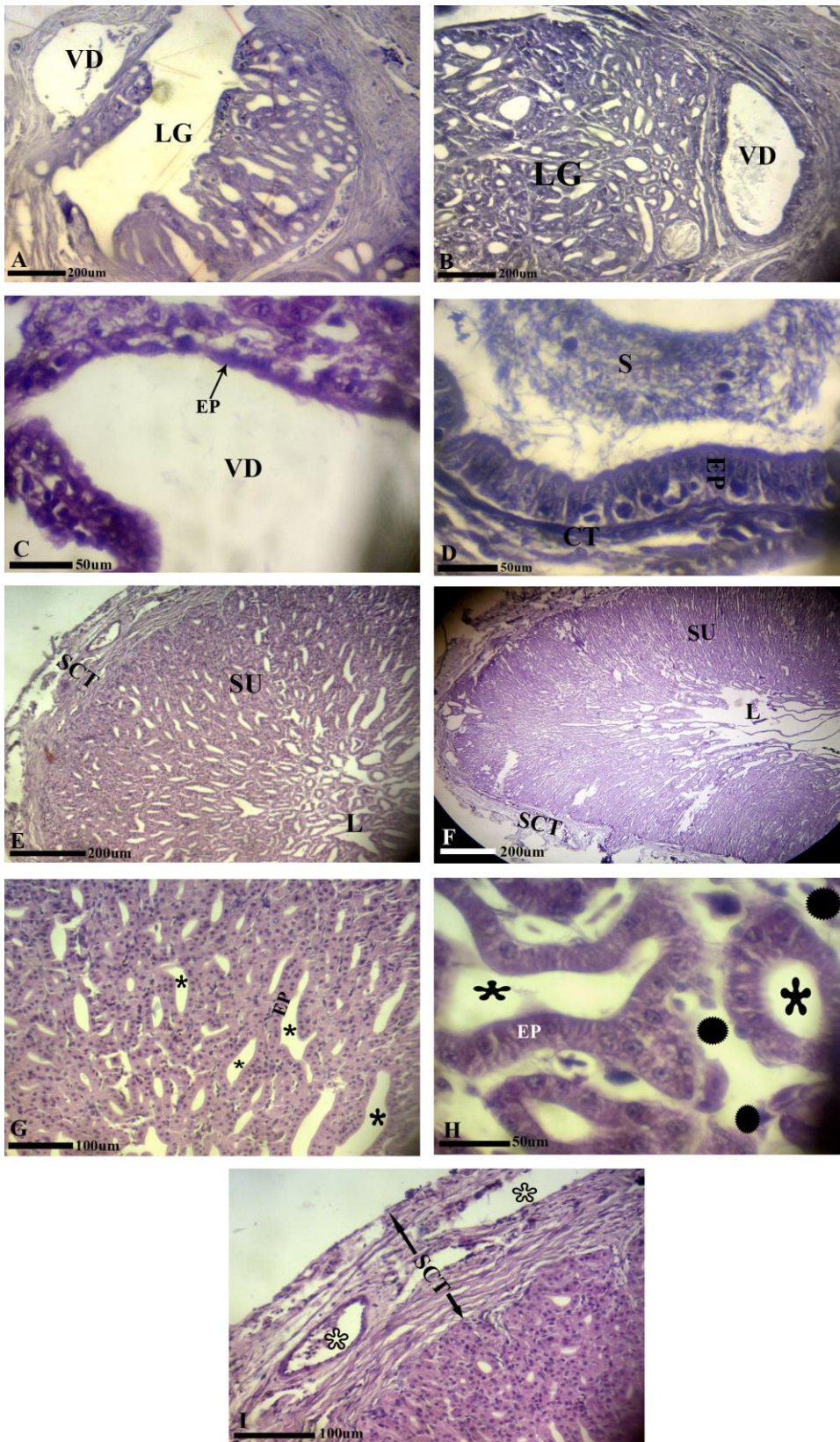


Fig. S6. Representative photomicrograph of the vas deferens and the Leydig gland in male *Chiloscyllium punctatum*; A,B. The vas deferens (VD) and the leydig gland (LG) in immature (A) and maturing (B) males; C. The vas deferens lined by a simple squamous epithelium in immature males; D. The vas deferens lined by a pseudostratified columnar epithelium in mature males; E,F. The leydig gland in maturing (E) and active (F) males; G,H. The tubular secretory units (black stars) of the Leydig gland coated by a simple cuboidal epithelium; I. Capsule of serous connective tissue (SCT) with many blood vessels (white stars) around the leydig gland; Epithelium (EP), Spermatozoa (S), serous connective tissue (SCT), secretory units (SU), lumen (L); A,B,E,F (H&E; 290×), C,D,H (H&E; 2900×), G,I (H&E; 725×).

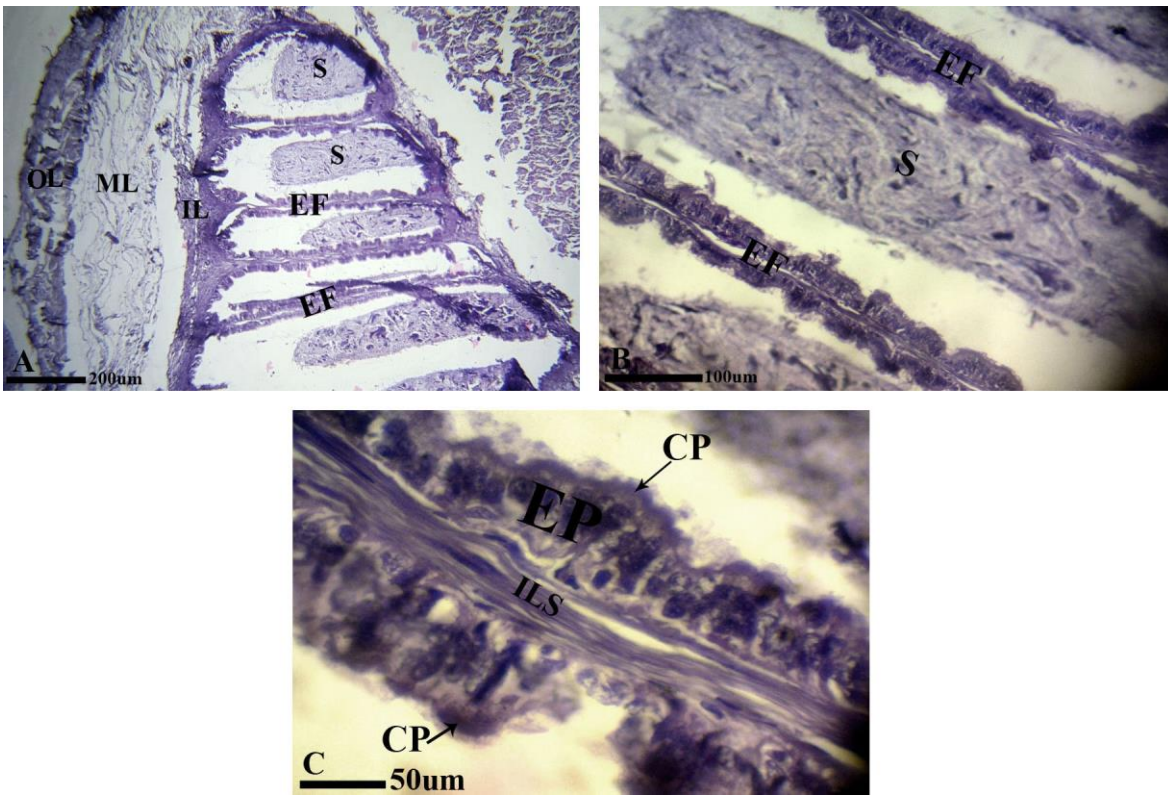


Fig. S7. Representative photomicrograph of the seminal vesicle in active mature male *Chiloscyllium punctatum*; A,B. The seminal vesicle: the extensive epithelial folds (EF) and the luminal secretions with spermatozoa (S). The seminal vesicle was coated by the three laminar capsule included outer (OL), middle (ML) and inner (IL) layers; C. The seminal vesicle folds coated by simple cuboidal epithelial cells, the cytoplasm of which was projected into the lumen (CP); cytoplasm projection (CP); epithelium (EP), epithelial folds (EF); outer layer (OL), middle layer (ML), inner layer (IL), inner layer septa (ILS), spermatozoa (S); A (H&E; 290 \times), B (H&E; 725 \times), C (H&E; 2900 \times).