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# Histological Studies on Some Organs of Two Male Dealfishes, *Trachipterus ishikawae*, Caught on the Beach of Shirahama, Wakayama Prefecture, Pacific Coast of Japan

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**Abstract** The testis, thyroid, kidney, exocrine pancreas, endocrine pancreas (=Brockmann body), spleen and liver of two dealfishes, *Trachipterus ishikawae*, collected on the beach of Shirahama, Wakayama Prefecture, Pacific coast of Honshu, Japan, were examined histologically. The specimens measured 2.30 m (specimen A) and 1.65 m (specimen B) in total length. The testicular lobes were atrophied and sclerotized, having the appearance of an aggregation of numerous protuberances (or spherules), each spherule corresponding to a lobule containing a number of cysts, although some of the latter had collapsed and/or merged with each other. Each cyst contained sperms and spermatids, relict sperms being found in the spermiduct. The thyroid gland, an amorphously soft body, located dorsally on the ventral aorta and its afferent branchial arteries, was composed of many variously-sized follicles. Each follicle comprised a large amount of smooth colloid, surrounded by a flat epithelium consisting of cubic cells. The overall condition indicated an inactive state. In the kidney, a great deal of stromatous tissue, including AF positive lipid grains, and a small number of glomeruli were apparent. In both specimens, the thread-like loose connective tissue comprising the exocrine pancreas attached to and running parallel with the liver, stomach, Brockmann body and spleen, were composed of acini with zymogen-rich pyramidal cells. Details of cell types in the Brockmann body were difficult to determine. Fatty deposits were absent from the liver of specimen A, which was characterized by rich stromatous tissue and dilated sinusoids. However, rich fatty deposits were found in several portions of the liver of specimen B. Well-developed stromatous tissue and nodules of red- and white pulps were recognized. It was concluded that the two specimens were spent and debilitated males.

**Key words**: visceral organ histology, testis histology, anatomy of organs, male dealfish, spent dealfish

## Introduction

On 12 January, 2004, a large male dealfish, *Trachipterus ishikawae*, was stranded on the beach of Saino-Kamoi, near Seto Marine Biological Laboratory (Kyoto University), Shirahama, Wakayama Prefecture (Kubota and Tanase, 2004). Subseuently, on 25 April, 2004, a second, smaller dealfish was caught in Tanabe Bay, opposite the above Laboratory. Both specimens were initially measured and dissected by SK, and the visceral organs (preserved in 10% formalin solution) forwarded to YH for detailed histological investigation so as to determine the degree of debilitation of several organs, in respect of the gonadal conditions (initially diagnosed as immature ovaries).

Reports dealing with the gonad maturity and architecture of other organ tissues in lampridiform (in particular the taeniosomate) are relatively few (Tamura and Honma, 1971,1972, Honma *et al.*, 1973; Honma and Tsumura, 1980; Honma and Mizusawa, 1981; Honma *et al.*, 1999, 2002; Kobayashi, 2004; Kobayashi *et al.*, 1996). Recently, Honma *et al.* (2004) desribed the ovarian histology of a large dealfish caught in a Kumano-nada Sea, Wakayama Prefecture, and two oarfishes entangled in a set net installed off the coast of Wakasa Bay, Sea of Japan. In addition, Honma *et al.* (2005) described the

testes histology of two dealfish individuals stranded on the coast of Niigata District, Sea of Japan.

However, histological examination carried out on visceral organs (including gonads) were insufficient to assume the reproductive biology and life habit of these rare and unusual taeniosomatous fishes. In order to add further information, the present specimens provided a good opportunity for histological comparison with the previously reported individuals.

## Materials and Methods

A large dealfish (specimen A), 2.30 m in total length and 0.35 m in depth (body weight unknown) was caught by Mr. T. Sedoh in a depth of 1 m near the beach of the Seto Marine Biological Laboratory (Kyoto University), Shirahama, Wakayama Prefecture, central part of the Pacific coast of Honshu, Japan. A smaller specimen (specimen B), 1.65 m length and 4.5 kg in body weight, was caught by Mr. J. Minami in a depth of 2 m near the beach at Tanabe Bay, opposite to the Laboratory. After died, both individuals were presented to the Laboratory, and, after a lapse of several hours for gross anatomy, their visceral organs were preserved in 10% formalin and subsequently being forwarded to the Anatomy Department, Niigata University Medical School. The dates of collection were 12 January, and 25 April, 2004, respectively.

Among the organs, the testis, thyroid, kidney, exocrine pancreas, endocrine pancreas (so-called Brockmann body), spleen and liver were selected and removed, a part of each organ being refixed with Bouin's solution. Pieces of each organ were dehydrated through an alcohol series, embedded in paraffin, cut in 5 $\mu$m sections, stained with hematoxylin-eosin (HE) double stain and Masson-Goldner (MG) associated with aldehyde fuchsin (AF) tetrachrome, and observed under a light microscope (Leitz Orthoplan).

## Results

### Macroscopy

**Testis**: The testis in specimen A was elongate, white in color, and measured 29.0 cm long, 3.0 cm wide and 75 g in weight. A smaller testis in similar condition in specimen B was 19.0 cm long, 1.5 cm wide and 6 g in weight. Significant degenerative changes were apparent in the external features of the testes, all lobules being atrophied and strongly sclerotized so as to appear as numerous protuberances (or spherules) with a suggestion of grainy bands (Fig. 1). Each protuberance was firm with some elasticity.

**Thyroid**: The brownish-yellow thyroid, located dorsally on the ventral aorta and its derivatives, primarily the 1st and 2nd branchial arteries, was removed from specimen A only, being some 10 mm in length, and having a long ovoid depressed disk-like shape. Larger follicles were recognizable with the naked eye.

**Kidney**: The tapeworm-like kidney of specimen A, dark red in color, was about 60 cm long and 30 g in weight.

**Exocrine Pancreas**: Exocrine pancreatic tissue was included in white thread-like connective tissue, along the right side of the posterior portion of the liver to the stomach. The Brockmann body and spleen were included in the same connective tissue (Fig. 1).

**Endocrine Pancreas (= Brockmann body)**: The Brockmann body was spherical, being attached to the right side of the posterior corner of the liver. It measured 4 mm long and 1 mm wide in specimen B, but was lost from specimen A during the initial dissection.

**Spleen**: An ovoid body, tinted dark red, which measured 7 mm (longer axis) by 5 mm (shorter axis) in specimen B (Fig. 1).

**Liver**: The hepatic organ, mud yellow in color, was a large, bilaterally asymmetric lobate mass.

### Histology

**Testis**: The testes of both specimens were enveloped by delicate mesothelial tunica vaginalis and

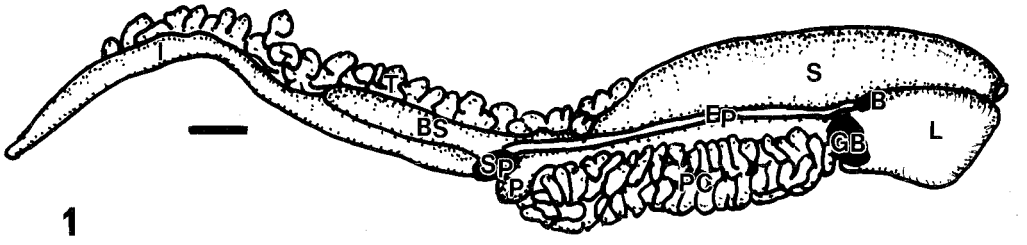


Fig. 1. Digestive organs associated with the protuberant testis of a spent dealfish (specimen B). B, Brockmann body; BS, blind sac of stomach; EP, exocrine pancreas; GB, gall bladder; I, intestine; L, liver; P, pylorus; PC, pyloric caeca; S, stomach; SP. spleen; T, testis. Scale bar: 2 cm.

tunica albuginea (Fig. 2). Most of the grainy featured lobules consisted of exhausted (vacant) cysts with or without relictual sperms. However, some of the lobules still retained numerous spermatids and sperms (spermatozoa) in the cysts. Many cystic walls were expanded and burst, some being united with each other to form a large lumen (Fig. 3). In the center of cysts comprising spermiogenetic cells (spermatids), a wide lumen was encountered (Fig. 4).

Between the cysts, small amounts of interstitial tissue remained. Neither Leydig nor Sertoli cells located near the cystic wall were apparent (Fig. 5). Noticeably, spermatogenetic cells (spermatogonia and spermatocytes) were not present in the lobules.

It was concluded that the testes of the two dealfishes were spent, in spite of their different individual body sizes, the spermatozoa being in similar developmental mode.

**Thyroid**: The thyroid gland of specimen A, buried in the loose connective tissue, was of a diffuse nature, and its structural components (follicles) being variable in shape and size (24~120 $\mu$m in longer axis) (Fig. 6).

The follicular epithelium, 6~12 $\mu$m in height, contained much homogeneous colloidal substance, which stained with eosin and AF, indicating a glycoproteinous nature. The epithelium of the largest follicles was flat, but consisted of cuboidal to very low columnar cells. The nucleus was situated basally, with a distinct large vacuole in the apical portion of each cell. However, neither droplets nor a wavy margin were apparent in the colloid (Fig. 7).

It was concluded that the gland was inactive or quiescent.

**Kidney**: Although only a small portion was removed and examined, good preparations were secured from specimen A. The distribution of segments of epithelial-lined-tubules and glomeruli was only moderately dense, whereas the amount of interstitial tissue was considerable (Fig. 8). It was difficult to discriminate between the cortex and medulla. However, the sections of both the proximal and distal convoluted tubules in the cortical (superficial) region included apically-located nuclei. In addition, the density of the proximal segments was greater than that of the distal segments (Fig. 9).

Due to the existence of minute rich organelles and inclusion bodies, a strong staining affinity was obvious in the cytoplasm of the proximal segments. Debris and tiny bubbles recognized in the lumen appeared to have been derived from collapsed ciliary tufts (= brush border), although the healthy tufts were still prominent and reacted positively with AF (Fig. 9). Similarly, owing to the existence of lipids, the interstitial cells were strongly positive to AF. Between the interstitial cells, numerous capillaries were encountered.

The macula densa, consisting of high columnar cells, was clearly apparent in the vascular pole of the glomerulus near the origin of the distal tubules (Fig. 9). Adjacent to the cell mass of the macula

densa, large cells comprising rich granules were diagnosed as juxtaglomerular cells (Fig. 9). However, in the present preparation, it was difficult to identify flat Goormaghtigh cells.

**Exocrine Pancreas**: This organ had a peculiarly cord-like structure consisting of a continuation of acini, a transverse section containing only two to five of the latter. Between the exocrine pancreas and the liver, a vein and connective tissue septa were visible.

Each acinus was composed of several pyramidal cells, each of which had a basally-shifted nucleus and an apically-shifted richly granular (droplets) portion. Although the nucleus and marginal part of the cytoplasm were stained by hematoxylin (or tinted red with phroxin), apical granules were stained deeply with eosin (or strongly positive with AF) (Fig. 10). One or two small centroacinar cells were recognized in the margin of the intercalated duct located in the center of the acinus. However, there were no notable structure in this tissue.

**Endocrine Pancreas (= Brockmann body)**: The Brockmann body was enveloped with a comparatively thick capsule of loose connective tissue containing exocrine pancreatic tissue. Due to heavy shrinkage during fixation, abundant lacunae had developed, and the endocrine cells had become wedge-shaped. Accordingly, only AF- positive B (insulin producing) cells and phroxine (or eosin)-positive A (glucagon producing) cells are demonstrated here (Fig. 11).

**Spleen**: In both specimens, the spleen, enveloped with a thick capsule of loose connective tissue comprising the exocrine pancreatic tissue, was composed of well-developed stromatous tissue, the density and aggregation of parenchymatous cellular components being low (Fig. 10). However, nodules and small, variously-sized masses, consisting of red blood cells (red pulp) and lymphocytes (white pulp) were encountered (Fig. 10). In the sheathed arteries, rod-shaped endothelial cells were defined. Splenic sinuses were also well-defined, although no melanomacrophages were recognized.

**Liver**: In both specimens, it was difficult to discriminate the central vein and radially-derivative sinusoids accompanying the radially disposed hepatic cell cords. In contrast to the typical structural pattern, a number of dilated veins and sinusoids were found distributed randomly throughout the hepatic lobe. Accordingly, the hepatic cells (polyhedral shape) were accumulated into numerous masses (Fig. 12).

Although no noteworthy features were identified in the hepatic cells using HE stain, MG-AF stain demonstrated AF positive material in some cells (Fig. 12). Most notable was a small amount of parenchymatous tissue (hepatic cells), while vascular and stromatous tissues were also evident.

Moreover, massses of hepatic cells with peripherally-depressed nuclei, containing huge fat droplets and cells with rich cytoplasm stained with eosin (or phroxin) were apparent (Fig. 13), the two cell masses being interwoven with each other.

## Discussion

In spite of the variably severe deterioration of organs and tissues of stranded taeniosomatous fishes (Lampridiformes), efforts have been made to accumulate and elucidate anatomical and histological evidence from time to time (Tamura and Honma, 1971, 1972; Honma and Tsumura, 1980; Honma and Mizusawa, 1981; Honma *et al.*, 1973, 1999, 2002, 2004). However, apparently only 3 papers have (briefly) touched on the macroscopic anatomy of taeniosomatous fishes (Vaissiere, 1917; Nishimura, 1963; Harrison and Palmer, 1968).

In Japan, a further report on the gonadal histology of taeniosomatous fishes was given as an oral presentation (Kobayashi *et al.*, 1996). Subsequently, Kobayashi (2004) compiled and listed the histological specimens of gonads comprising 349 species of fishes. In this list, he presented 33 preparations belonging to 7 species of taeniosomatous fishes accompanying 4 colored microphotographs (3 ovaries and 1 testis) of oarfishes. Using these specimens, Kobayashi *et al.* (1996) briefly mentioned that in male fishes spermatogonia existed locally in each cyst, there being no indication of intersex recognized, while in female fishes, the entovarian type of ovarian structure and unequal developmental mode of ovarian eggs were noted.

In the above studies by Honma and associates on gonadal and other organ histologies of 14 specimens representing 5 species, 5 male individuals, including the present specimens, have been examined, in addition to 8 females and one sex unknown. Among the 5 males, one was immature and 4 were spent (Honma and Mizusawa, 1981; Honma *et al.*, 2004, and unpublished data).

The testes of the present dealfishes have become remarkably degenerate and atrophied, having grainy protuberances comprising expanded and/or burst cysts with spermatids and spermatozoa, at the same time lacking spermatogonia and spermatocytes. These features and the microscopic evidence led to the conclusion that the testes were spent and senescent, pointing to likely failure of any subsequent reproductive ability. Recently, 2 male dealfishes, smaller than the present specimens, stranded on the coast of Niigata District, Sea of Japan, were examined also found to be in spent condition (Honma *et al.*, 2005). However, the condition of the testicular lobules indicated some possibility of further reproductive success.

Examination of the thyroid gland, rarely reported in taeniosomatous fishes, provided a good comparison with earlier reports by Honma and associates, made on poorly-fixed material. Tamura and Honma (1971, 1972) and Honma *et al.* (1973), illustrated rough sketches of the gland and described microscopic features, including notes on the variously-sized follicles (70~80 $\mu$m [smaller] and 300 $\mu$m in [larger]), uniform height of the epithelium and rich smooth colloid. They mentioned that these features were diagnosed as being in hypofunctioning state, the present specimen confirming those observations. In order to elucidate real thyroid gland function, it is necessary to demonstrate the existence of thyrotropic cell activity in the hypophysis (if well- preserved material can be secured).

Although we lacked comparative histological figures of the kidney in taeniosomatous fishes, the present account should be a valuable addition to the biology of these fishes. The demonstration of AF-positive materials in the interstitial cells is thought to show the existence of proteinous glycosaminoglycans (= acid mucopolysaccharides). However, the function of the hyperplastic interstitial cells is unknown.

Although Nishimura (1963) described and illustrated the gross anatomy of a female dealfish, he did not identify the exocrine pancreas or Brockmann body. In spite of improper fixation, the histological architecture of the exocrine pancreas provided here is an improvement on previous reports (Tamura and Honma, 1971, 1972; Honma *et al.*, 1973, 2002). Dealing with a spent female dealfish, Tamura and Honma (1972) found a considerable number of regressive acini, every cell having a large vacuole in the basal portion. The present specimens showed no such a feature.

It is difficult to explain the differing histological condition of the Brockmann bodies of previous specimens (Tamura and Honma, 1972; Honma *et al.*, 1973), and a razorback scabbardfish (Honma *et al.*, 2002) compared with the present material. Therefore, further detailed studies are needed to determine the nature of secretory cells and state of the stromatous tissues.

An increase in stromatous tissue in the spleen was noticed not only in previous studies (Honma *et al.*, 2002), but also during the present examination, indicating decline of hemopoiesis.

A fatty liver, comprising a rich accumulation of fat droplets in the hepatic cells has been described previously (Tamura and Honma, 1972; Honma *et al.*, 2002). One of the present specimens was also characterized by fat accumulation, but also had a relatively large amount of stromatous tissue.

The histological data, derived from several organs, indicated that the present dealfishes were in the process of increasing senescence, with no likelihood of further reproductive ability, with the further implication of a short lifespan after final spawning. However, there is as yet little cytohistological information on the organs and tissues of taeniosomatous fishes, a situation that should be rectified as more of these rare and curious abyssal fishes come to hand.

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## Explanation of Plates

### Plate III. Figs 2-7. Microphotographs of dealfish visceral organs.

Fig. 2. Part of protuberant lobules of spent testis comprising numerous cysts (C) with or without spermatids and sperms. Hematoxylin-eosin (HE) stain. Scale bar: 50 $\mu$m.

Fig. 3. Cysts comprising relict sperms (arrows). HE. Scale bar: 25 $\mu$m.

Fig. 4. Cysts comprising entirely spermatids (arrows). Note expanded and burst cystic walls (W). HE. Scale bar: 2 $\mu$m.

Fig. 5. Cysts comprising spermatids (arrow heads) and sperms (arrow). Neither Leydig cells in the connective tissue nor Sertoli cells inside the cystic wall are apparent. HE. Scale bar: 25 $\mu$m.

Fig. 6. Thyroid gland buried in fibrous connective tissue around the ventral aorta. Follicles of various sizes surrounded by flat epithelia (arrows), the follicular lumen containing rich and smooth colloid. HE. Scale bar: 50 $\mu$m.

Fig. 7. A typically round follicle surrounded by low epithelium (arrows) consisting of cubic cells. Note basally-shifted nucleus and apically-shifted vacuole in each cell. HE. Scale bar: 25 $\mu$m.

### Plate IV. Figs 8-13. Microphotographs of dealfish visceral organs.

Fig. 8. Part of kidney showing distal convoluted tubules existing in copious interstitial tissue (I). HE. Scale bar: 25 $\mu$m.

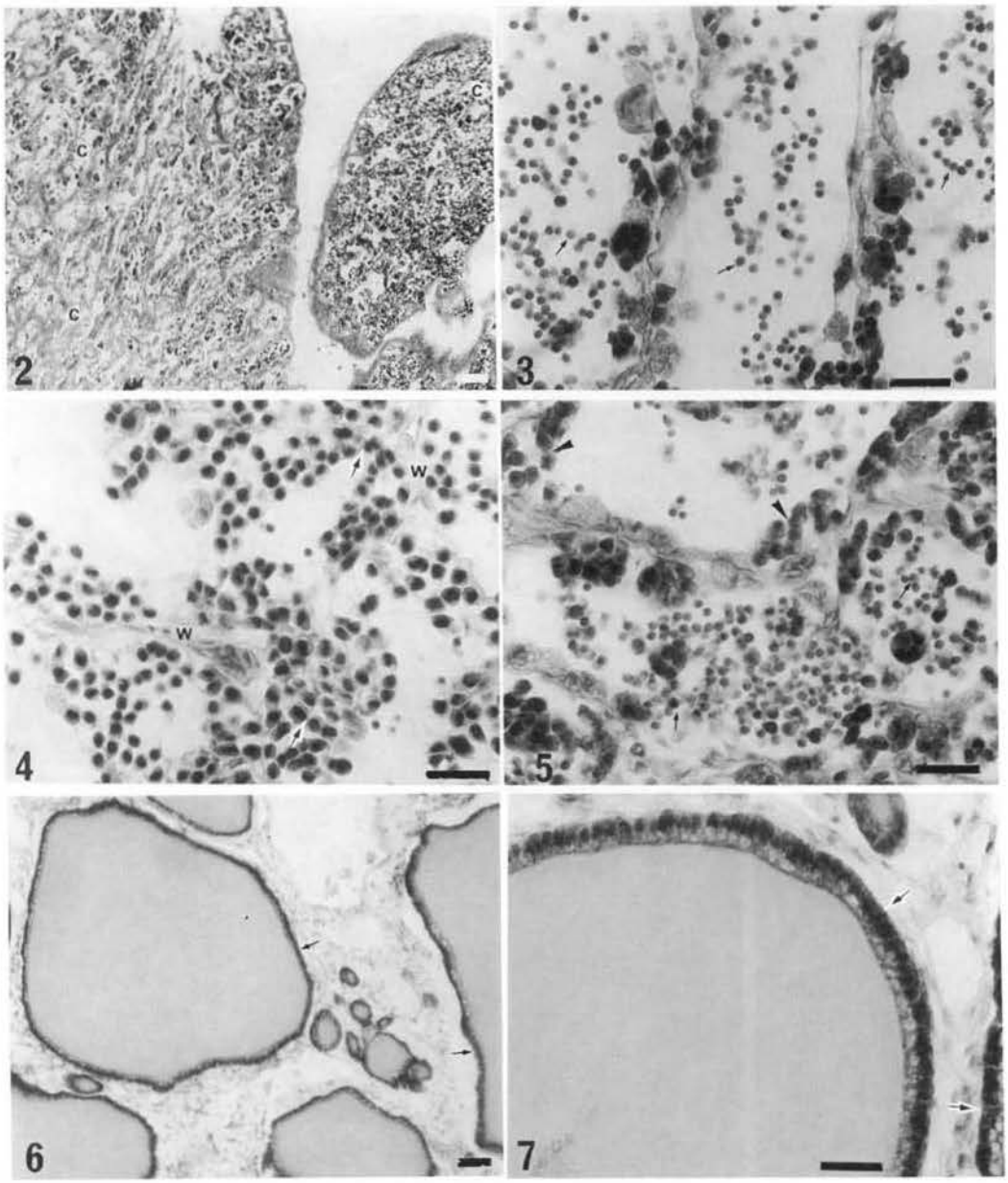
Fig. 9. Cross section of proximal convoluted tubules consisting of low columnar cells with apically-shifted nuclei and brush border (small arrow). Note a glomerulus showing capillary tufts (arrow), a vascular pole (arrow head) and macula densa (M). HE. Scale bar: 25 $\mu$m.

Fig. 10. Exocrine pancreatic tissue consisting of acini within a connective tissue band. Each acinal cell contained rich zymogen granules (arrow). Note spleen (SP) surrounded by a connective tissue capsule. HE. Scale bar: 25 $\mu$m.

Fig. 11. Part of Brockmann body showing AF-positive B (insulin producing) cells and phroxine-positive A (glucagon producing) cells. Note heavy shrinkage of endocrine cells and well developed vacuoles (v). MG-AF. Scale bar: 25 $\mu$m.

Fig. 12. Section of liver showing an irregular arrangement of pyramidal hepatic cells. a, HE; b, MG-AF, demonstrating AF-positive materials in the cells (arrows). Scale bar: 25 $\mu$m.

Fig. 13. Part of fatty liver showing well-developed fat droplets (F) in the hepatic cells. MG-AF. Scale bar: 25 $\mu$m.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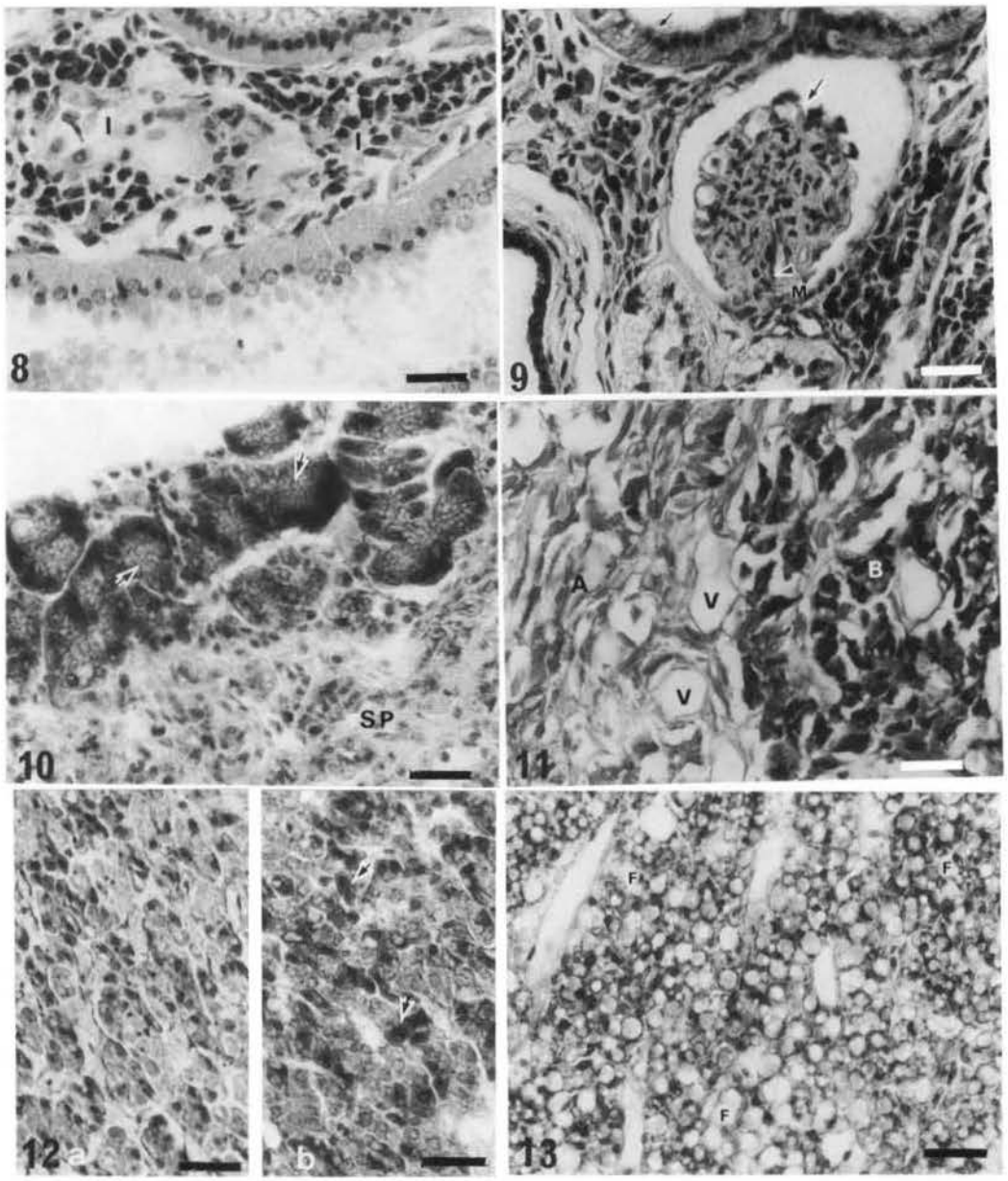
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