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Original Article

Light microscopic study of the pancreas in silurid catfish, *Eutropiichthys vacha* (Hamilton, 1822)

Saroj Kumar Ghosh*

Fisheries Laboratory, Department of Zoology, Bejoy Narayan Mahavidyalaya, Itachuna, Hooghly-712 147, West Bengal, India.

Abstract: The cellular organization of the pancreas in *Eutropiichthys vacha* was characterized using histological and histochemical techniques. The pancreas was a diffuse organ located near the stomach, composed of exocrine and endocrine tissues. The main mass of the pancreas consisted of exocrine tissue, within which clusters of endocrine cells, interspersed with blood sinuses and encased in a thin capsule, were scattered and formed islets. The exocrine pancreas was composed of serous acini and duct system, which were concerned with the synthesis and transport of digestive enzymes into the gut. In contrast, pancreatic islets, made up of alpha, beta, and delta cells, control the production and release of peptide hormones into the bloodstream. The acinar cells, densely packed with zymogen granules, indicated an intense tryptophan reaction, implying the presence of precursors for various pancreatic enzymes associated with the fish's feeding behaviour.

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Introduction

The pancreas, a compound racemose gland, consists of structurally distinct exocrine and endocrine components that play essential roles in digestion and metabolism. In most teleosts, the pancreas is typically diffuse rather than a distinct, easily recognizable organ. The anatomy and organization of the pancreas in ray-finned fishes vary widely, often dispersed throughout the peritoneal cavity, depending on their specific adaptations and specializations. Rizkalla (1967) classified the pancreas of teleosts into three types: compact, disseminated, and intrahepatic. In the catfish *Clarias gariepinus*, the pancreas is compact and embedded within the mesenteric adipose tissue between the liver, spleen, and stomach (Karkit et al., 2021). In ray-finned fishes, the pancreatic tissue is disseminated throughout the mesenteries and the coils of the intestine (Chanet et al., 2023). It is often associated with the esophagus, stomach, spleen, gall bladder, and pyloric caeca (Chakrabarti and Ghosh, 2015) and may even extend into the liver around the portal veins, forming the hepatopancreas (Mokhtar, 2015; Abusrer and Shtewi, 2023).

The exocrine pancreas is a lobulated organ composed of acinar cells organized into glands, along with a network of ducts that transport the digestive enzymes secreted by these cells into the small intestine (Yee et al., 2005). The endocrine pancreas consists of isolated masses of cells that are richly vascularized and have been referred to by various terms in the literature, including Brockmann bodies, islets of Langerhans, insular organ, islet tissue, and principal islets (Kaptaner, 2019). The islet tissue is composed of well-defined cell types, each responsible for producing a specific hormone. In osteichthyes, immunohistochemistry studies have identified four cell types: A cell, which produces glucagon; B cell, which produces insulin; D cell, which produces somatostatin and F cell, which produces pancreatic peptides (Youson et al., 2001; Lee et al., 2001; Kong et al., 2002; Fortin et al., 2015). However, these cell populations appear to be quite variable among teleost species, particularly in the case of F cells.

Considerable variations in pancreatic tissue, glandular structural components, and the differing arrangements of endocrine cells in teleosts present a

*Correspondence: Saroj Kumar Ghosh
E-mail: saroj.fisherylab@gmail.com
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fascinating area of study. These differences could offer insightful information about how the anatomy and function of this endodermal organ relate to one another. The present investigation focuses on the distribution and structural components of the pancreas, with particular attention to the characterization of cell types within the islets of the Schilbid catfish, *Eutropiichthys vacha*. This river catfish is highly predatory, primarily feeding on aquatic insects, crustaceans, arachnids, shrimp, annelids, small forage fishes, and macrophytes (Azadi et al., 1991; Abbas, 2010).

Materials and Methods

Specimen collection: Mature specimens of *E. vacha* (23.17±3.46 cm in total length; n = 12) were collected from the Bhagirathi-Hooghly River at Kalyani and its adjoining areas in West Bengal throughout 2023 using traditional fishing gear. After collection, the specimens were anesthetized with an overdose of 2-Phenoxy ethanol ($C_8H_{10}O_2$). A mid-ventral incision was made to open the body cavity, and pancreatic tissues were excised from the stomach wall for subsequent studies.

Histology: The pancreatic tissues were fixed in aqueous Bouin's fluid for approximately 18-24 hours. After proper washing in 70% ethanol, the samples were dehydrated through a graded series of ethanol and cleared with xylene. The tissues were then embedded in paraffin wax at 56-58˚C using a thermostat-controlled vacuum paraffin embedding bath for 1½ hour. Serial paraffin sections were cut at 4 µm thickness using a Weswox MT-1090A rotary microtome. Following routine procedures, the dewaxed sections were stained with Heidenhain's azan (HA) (Kiernan, 2008), Mallory's triple (MT) (Mallory, 1936), and Masson trichrome (M) (Masson, 1929) stains.

Histochemistry: Tissue samples were fixed in 10% neutral formalin for 16-18 hours. After proper dehydration through an ascending series of ethanol and subsequent clearing in xylene, the tissues were embedded in paraffin wax at 52-54˚C. Paraffin blocks were then cut into 8 µm thick sections and subjected to the p-Dimethylaminobenzaldehyde (DMAB)-nitrate method for the detection of tryptophan (Adams, 1957). The stained sections were mounted with DPX and observed under a Zeiss Primo Star light microscope, equipped with a Tucsen 5.0 MP camera.

Results and

Histological organization: In *E. vacha*, the pancreas is diffuse and located in the mesentery supporting the esophagus, stomach, and duodenal loop. Most of the pancreatic tissue is concentrated on the stomach wall (Fig. 1A). The pancreas is a mixed gland, containing both exocrine and endocrine tissue, enveloped by a thin layer of loose fibrous tissue (Figs. 1A-B). The exocrine portion, which constitutes the majority of the pancreas, consists of densely packed serous acini organized into small lobules (Figs. 1B, D). The lobules are enclosed by loose connective tissue that contains blood vessels, nerves, interlobular ducts, and infrequently Pacinian corpuscle. The pancreatic acini consist of pyramid-shaped secretory zymogenic cells surrounding a small central lumen (Fig. 1D). The rounded nuclei are located in the broader basal portion of the acinar cells, while acidophilic zymogen granules are present in the apical part. The secretions exit the serous acini through intercalated ducts, which have narrow lumina lined with cuboidal epithelium (Fig. 1C).

The endocrine pancreatic islets are distinguished from the surrounding exocrine acini by a thin capsule layer, which contains reticular fibers (Figs. 1A-C, E-F). They are scattered among the acini, arranged in clumps, and are richly supplied with blood capillaries. Three principal islet cell types: alpha, beta and delta cells-are identified by their distinct staining characteristics, cytoarchitecture and distribution within the islets. Alpha cells are relatively numerous and oval in shape, characterized by deeply stained nuclei and coarse granules that are colored with acid fuchsin (Fig. 1E). They are generally located at the periphery of the islets. Beta cells are more numerous and aggregated in the middle part of the islet. They are more or less round or irregular in shape, with a centrally located nucleus and cytoplasmic ground

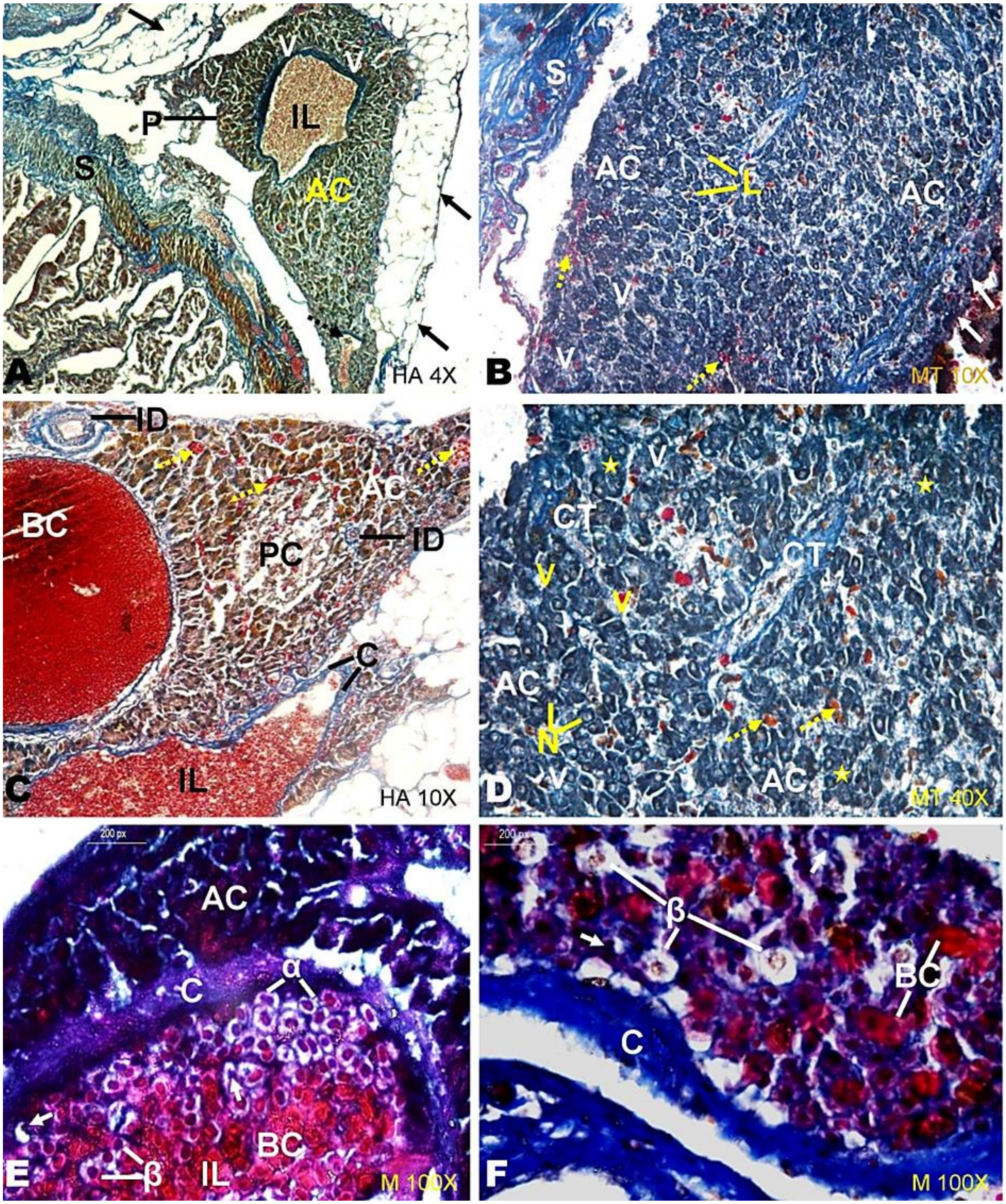

Figure 1. Photomicrographs of pancreatic sections in *E. vacha* stained Heidenhain's azan (HA), Mallory's triple (MT) and Masson trichrome (M) stains. **(A)** Pancreatic tissue (P) enveloped with connective tissue (solid arrows) and affixed to stomach wall (S) consists of bunch of acinar cells (AC). A thin connective tissue capsule (arrow heads) separates the islet of Langerhans (IL) from serous acini. **(B)** The exocrine pancreas consists of clusters of AC arranged into lobules (L) surrounded by connective tissue septa (arrowheads) that contain blood vessels (broken arrows). Loose connective tissues form a capsule (solid arrows). S marks the stomach wall. **(C)** IL is demarcated from exocrine AC by athin capsule (c). Fine blood vessels (broken arrows), intercalated ducts (ID) and Pacinian corpuscle (PC) are observed among the acini. Note the presence of blood capillaries (BC) around IL. **(D)** AC packed with zymogen granules (asterisks) are arranged into small lobules around a central lumen (arrow heads), surrounded by interlobular connective tissue septa (CT) containing blood vessels (broken arrows). N for Nuclei of AC. **(E)** IL is separated from surrounding AC by a connective tissue capsule (C) contains α cells, β cells and δ cells (arrow). Patches of blood cells form capillary network (BC). (**F)** Enlarged view of IL bounded by C contains β and δ cells (arrows) adjacent to BC.

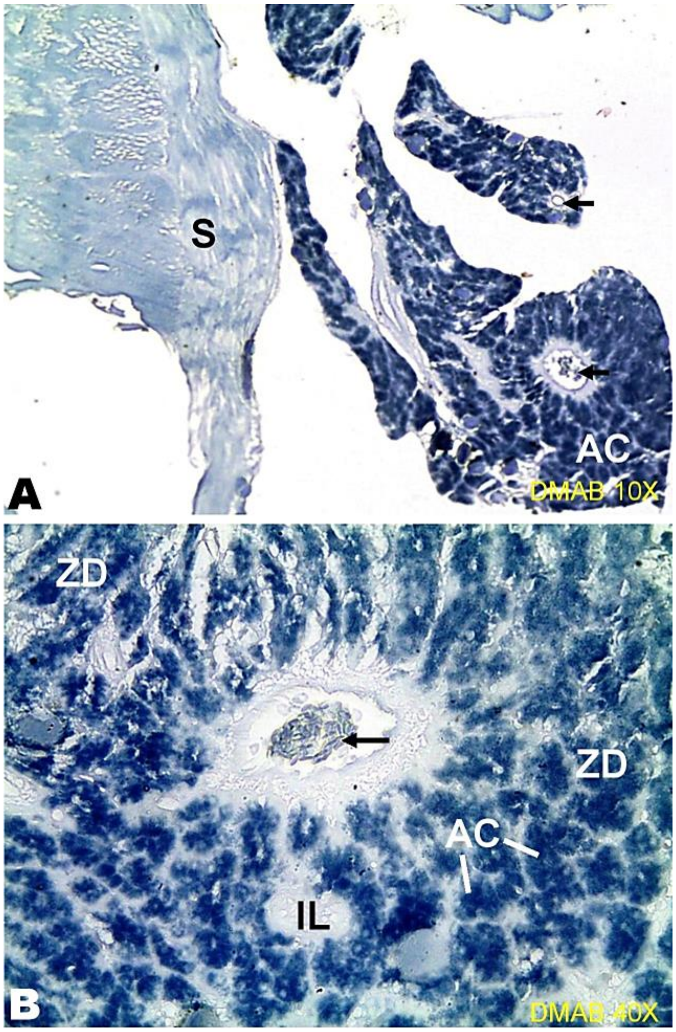

Figure 2. Microphotographs of pancreatic sections in *E. vacha* display the histochemical localization of trypotophan (DMAB). **(A)** Shows an acute tryptophan reaction in the acinar cells (AC) of pancreatic tissue attached with wall of stomach (S). Blood cells (arrows) show a moderate reaction. **(B)** Shows a strong tryptophan reaction in the zymogen droplets (ZD) of AC. Blood cells (arrows) exhibit moderate reaction, while the islet of Langerhans (IL) show a faint reaction.

materials stained with Masson trichrome (Fig. 1F). The nuclear membrane and nucleolus of beta cells are evident and precisely marked. Delta cells are scanty in number and located at the periphery of the islets. They vary in shape and contain elliptical nuclei and cohesive cytoplasm stained with bluish-purple colour (Figs. 1E-F). The blood capillaries encompassing the endocrine cells show the extensive vascularization of the islets of Langerhans.

Detection and localization of tryptophan: The serous secretory cells of the acinus exhibit an intense blue color, indicating a positive reaction for tryptophan (Fig. 2A). Similarly, the zymogen droplets in the acinar cells of the exocrine pancreas, responsible for synthesizing and secreting digestive enzymes, show a strong tryptophan reaction (Fig. 2B). In contrast, the islets of Langerhans in between the acinar tissue display a faint reaction. Additionally, the blood cells in the capillaries show a moderate tryptophan reaction (Figs. 2A-B).

Discussions

The digestive tract and associated glands of fish have adapted and evolved in response to the nature of their food and feeding habits. The pancreas of fish, like that of all vertebrates, consists of two primary components: an exocrine component and an endocrine component. In most teleosts, the pancreas is widespread and can be found in various areas depending on the species. It can be located within the mesenteries, spleen, liver, between the pyloric caeca, on the surface of the stomach, intestine, or around the portal system. It can also be imbedded in fatty tissue. In contrast, the pancreas adopts a more compact and leafy form in higher vertebrates. According to Gonzalez et al. (1993), *Serranus cabrilla* has a diffuse pancreas that develops islets inside the connective tissue surrounding some digestive organs and extends across the mesentery. In the present study, the pancreas of *E. vacha* is dispersed in nature and is located on the surface of the stomach. Similar results have also been observed for *Mystus vittatus* (Chakrabarti and Ghosh, 2015) and *M. gulio* (Ghosh and Chakarabarti, 2016).

The exocrine pancreas of *E. vacha* is made up of clusters of acinar cells that are densely packed with zymogenic granules, which are regarded as secretory granules, according to the current study. Precursors of many pancreatic digesting enzymes, which are secreted into the pancreatic duct in an inactive state, are found in these granules (Eroschenko, and di Fiore, 2013). The exocrine pancreas secretes water, bicarbonate, and other ions in addition to digesting enzymes. Via a system of ducts, the digestive secretion enters the intestine. Proenzymes, which are present in zymogen granules in acinar cells, are in charge of breaking down proteins, carbohydrates, lipids and nucleotides (Mokhtar, 2015). *Eutropiichthys vacha* has a system of ducts that allow the digestive secretion to enter the intestine and aid in the efficient digestion of proteinaceous foods. Protease, amylase, lipase, deoxyribonuclease, and ribonuclease are among the enzymes released by exocrine acini that are identical to those found in other vertebrates (Buddington and Kuźmina, 2000). The type of diet the fish eats is related to the relative amounts of several enzymes. The serous acini not only secrete a wide range of enzymes but also generate bicarbonate, which balances other electrolytes, including gastric acid. According to Field et al. (2003), the zebrafish's exocrine pancreas generates a number of digestive enzymes, such as trypsin, amylase and carboxypeptidase A, to aid in the efficient breakdown of food.

The alpha, beta and delta cells of the pancreatic islets of *E. vacha* are in contact with blood sinuses and scattered throughout the exocrine acini. These islets are encircled by dense fibrous connective tissue fibers. Nonetheless, reports of the fourth cell type, PP cells, in certain fishes, have been made (Lee et al., 2001; Fortin et al., 2015). It was observed that in the principal islets, alpha (A) cells are primarily located at the periphery, beta (B) cells are concentrated in the center, and delta (D) cells are distributed throughout the islet structure. Other teleost species, like *Ctenopharyngodon idella* (Mokhtar, 2015) and *Alburnus tarichi* (Kaptaner, 2019), also exhibit a similar distribution of islet cells. Endocrine cells have been seen to come into touch with blood sinuses, and occasionally, their secretory contents have been detected. A, B and D cells of the endocrine component of the pancreas in teleosts are in touch with the capillaries, also observed by Iaglov (1978). Emiocytosis is the process by which these endocrine cells extrude their hormones. Moreover, a number of metabolic variables, the eating state, and environmental factors all affect pancreatic secretion. The majority of teleosts primarily produce insulin, which is produced in the beta cells of the pancreatic islets, whereas the alpha cells produce glucagon. According to Hsu and Crump (1987), both hormones control blood serum glucose levels. Insulin, which is released from beta cells in response to elevated blood glucose, allows glucose to enter body cells where it is metabolized into energy. Insulin promotes gluconeogenesis in the liver and skeletal muscle by increasing their absorption of glucose. Insulin also promotes lipogenesis and improves fatty acid uptake in the liver (Plisetskaya and Duguay, 1993). On the other hand, when blood glucose levels are below normal, glucagon promotes the release of glucose into the blood and works primarily against insulin. It causes the glycogen that has been stored in the liver to break down into glucose, which raises the amount of sugar in the blood that leaves the liver (Plisetskaya et al., 1986). Somatostatin, which is secreted by delta cells, suppresses the release of insulin, glucagon, motilin, cholecystokinin, gastrin, secretin, and stomach acid (Kitamura et al., 1984). Furthermore, somatostatin prevents the gastrointestinal system from absorbing glucose, fatty acids, and amino acids (Lee et al., 2001). Moreover, hyperglycemia and lipolysis are induced by somatostatins (Sheridan et al., 2000).

In *E. vacha*, the exocrine acinar cells are more abundant than the endocrine cells and can be identified by p-dimethylaminobenzaldehyde, which detects the presence of zymogen granules. Tryptophan, a precursor of pancreatic enzymes, is secreted into the lumen of serous acini and is mostly related to the zymogen granules of the acinar cells. The synthesis and secretion of digestive enzymes in varied proportions, which reflect the fish's feeding patterns and dietary requirements, may be interrelated to the variable intensities of tryptophan content in the acinar cells (Chakrabarti and Ghosh, 2015). Electron microscopy and immunohistochemistry are required for further study on the pancreas of *E. vacha* in order to identify the cellular elements of the principal islets and ascertain their meticulous physiological significance.

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