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Comparative scanning electron microscope studies of hepatic parenchymal architecture in the three infradivisions of teleosts

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Abstract This report advances the phylogenetic hierarchy in teleosts with regard to micro-hepatic architecture. To demonstrate the correlation between liver structures and phylogenic status, we analyzed livers from nine teleost taxa using light and scanning electron microscope and subjected the data to phylogenic analysis. We observed that the hepatocytes were spread out as anastomotic cords, arranged in two to several cellular layers, and surrounded by sinusoids. We also found that in many species within the three infradivisions, hepatocyte nuclei were not centrally located, unlike the hepatocyte nuclei of mammals. The parenchymal arrangement evolved in parallel with phylogenic advancement. As phylogenic branching is graded in ascending order from the primary or secondary type to the final level, the parenchymal arrangements progressed from solid or tubular to cord-like, and the shape of hepatocytes changed from round to square and/or polyhedral. Thus, the hepatic lobular structures of the Euteleostei livers were more complete and of an advanced type, similar to that of mammalian livers. A phylogenic study of fish may be valid as an optimal model for liver ontogeny in vertebrates.

Keywords: evolution, liver, morphology, phylogeny, teleost

Introduction

The hepatic architecture comprises two tissue compartments, the epithelial cells that perform the organ's major functions (parenchyma) and the blood vessels and connective tissue (stroma). The parenchyma includes various cell types situated within the liver as well as the respective extra-cellular spaces, and the stroma includes the major vessels that nourish the liver, namely the portal vein and the hepatic artery. The liver of fish is a dense organ ventrally located in the cranial region of the general cavity, and its size, shape, and volume are adapted to the space available among other visceral organs. In many teleost species, the liver is divided into two or three lobes. However, lobulation is not evident in some teleost fish. In mammals and other vertebrate animals, hepatic plates, called one-cell-thick plate, line the simple-layered hepatocytes and pass from the portal triad to the central vein located in the center of the hepatic lobule. The hepatic parenchyma in fish forms two to several cellular plates surrounded by sinusoids. Between two neighboring sinusoids, hepatocyte-sinusoidal structures are arranged as tubular or solid cords that are generally two to several cells thick (Akiyoshi and Inoue 2004). The plate structures extend between central and portal zones.

Reports on the morphology of teleost liver reflect some controversy in data interpretation regarding parenchymal micro-architectures and confirm interspecies variations (Figueiredo-Fernandes et al. 2007; Hardman et al. 2007). Moreover, hepatic architecture normally varies with endocrine changes influenced by environmentally-regulated breeding status and in direct relationship to sex, age, nutritional status, and temperature. Hepatocytes, the major cell type in the liver, have been the primary subject of liver studies, but there are other important fundamental elements of fish-liver architecture to be examined. Only a few studies have addressed the question of the organization of stromal elements in fish liver and adequately advanced histological nomenclature differing from that used for mammalian liver.

In parenchymal cells of normal human and rat liver, the

nuclei are ordinarily located near the center of the cell, and this location indicates an important modulator for proper physiological liver function (Sato et al. 2001).

Materials and Methods

Sample collection

For this comparative morphological study, the livers of 9 different teleost species were used. We collected 8 species from ponds and streams in Shimane Prefecture, 1 species in Iriomote Islands in Okinawa Prefecture. In order to eliminate the influence of seasonal changes or growth, all specimens were caught from April to October in each locality from 2008 to 2011. Three to five specimens were sampled. Animals were anesthetized by immersion in an ice water bath in 2ml/L aqueous ethylene glycol monophenyl ether (Merck). After deep anesthetization, liver was taken from the animal. The phylogenetic relationships of teleost Class, comprising the nine teleost species from three infradivisions listed in Table 1.

Table 1
The study used the following nine teleost species belonging the three infradivisions, which was listed in the table 1:

Infradivision	Order	Species
Elopomorpha	Anguiliformes	1. *Anguilla japonica* 2. *Gymnothorax pictus* 3. *Conger japonicus*
Otocephala	Cypriniformes	4. *Cyprinus carpio* 5. *Ischikauia steenackeri*
	Siluriformes	6. *Plotosus lineatus*
Euteleostei	Perciformes	7. *Lateolabrax japonicus* 8. *Parapristipoma trilineatum* 9. *Girella punctata*

Light microscopy.

The liver samples were perfusion-fixed via the portal vein with 4% paraformaldehyde in phosphate buffer at pH 7.4 for 10 min, cut into small pieces, and immersed in the same solution for 3 days at 4℃. The specimens were rinsed, dehydrated in high-grade ethanol, and embedded in paraffin (SAKURA, Japan). Serial sections (4 μm thick) were stained with hematoxylin-eosin and analyzed and documented photographically with an Olympus microscope (Japan).

Scanning electron microscopy.

Liver fragments were processed by the osmium-tannic acid-osmium method (Akiyoshi and Inoue 2004). Briefly, samples were washed with phosphate buffered saline (PBS) and fixed with 2% glutaraldehyde in 0.1 mol/l phosphate buffer (pH 7.4) for 30 min at room temperature. The samples were then washed with the same phosphate buffer and post-fixed with 1% osmium tetroxide in the phosphate buffer. They were subsequently stained with 4% tannic acid and 1% osmium tetroxide. After dehydration in a series of graded ethanol solutions, they were freeze-dried with t-butyl alcohol. The samples were set in silver paste on a metal stage (Nisshin EM Co., Tokyo, Japan), and then spatter-coated with a layer of Pt-Pd, using a Hitachi E-1030 apparatus. The samples were finally examined with a scanning electron microscope (Hitachi S-8000, Japan).

Results

In each species, the liver was located ventrally in the cranial region of the body cavity and did not exhibit recognizable lobulation. The livers were typically reddish-brown in color and whitish after fixation, revealing rich vascularization. Hematoxylin-eosin staining facilitated discrimination of the hepatic parenchyma from the stromal elements; the stromal elements appeared as well-delimited connective tissue zones, often with embracing pancreocytes, or venous, and/or arterial vessels, and/or biliary ducts. The traditional lobulation, typical of mammalian liver, was not evident, as tracts and septa did not outline parenchymal areas. The connective tissue also harbored both pigmented macrophages and eosinophilic granulated (mast) cells. The histology of the parenchyma showed the hepatocytes forming a continuous cell mass, tunneled by sinusoids, resulting in a meshwork of two to several cell plates. Light and scanning electron microscopic images of liver samples from the nine teleost species showed great variety, unlike images of mammalian liver. Morphological analysis of livers from three Teleostei infradivisions revealed the following results.

Elopomorpha.

Hepatic lobule: Glisson's sheath: The hepatic lobule con-

sisted of a continuous field of connective tissue, the Glisson's sheath, enclosing the bile duct and arterial vessels in *Conger japonicus* (Figs. 1h and 2g). However, the connective tissue layers surrounding the portal vein were nearly absent or less developed in *Anguilla japonica* and *Gymnothorax pictus* relative to *C. japonicus,* thus the structures were incomplete. (Figs. 1a–c and 2d–f).

Sinusoidal structure: The highly branched sinusoidal capillaries were narrow and irregularly shaped; they appeared throughout the interstice between the hepatic plates (Figs. 1 and 2). These capillaries were highly distributed and branched and positioned nearer to the central zone than the portal zone.

Otocephala.

Hepatic lobule, Glisson's sheath: The hepatic lobule was a continuous, compact field of connective tissue surrounded by hepatocytes. The bile duct was located separately in the hepatic lobule in common carp, *Cyprinus carpio* (Fig. 4a). In contrast, the bile duct accompanied the venular vessels in striped eel catfish, *Plotosus lineatus* (Fig. 4g).

Sinusoidal structures: The sinusoidal structures were narrow and tubular and surrounded with two layers of polyhedral nucleated hepatocytes. The nuclei were usually small and located near the sinusoidal area in *C. carpio* (Fig. 4b, e, and h).

Euteleostei.

Hepatic lobule, Glisson's sheath: The hepatic lobule comprised plates that were two cells thick. In large-scale blackfish, *Girella punctata* (Figs. 5h and 6g) and Japanese seabass, *Lateolabrax japonicus* (Fig. 6a–c), the bile ducts accompanied a portal venule and hepatic arteriole similar to portal tracts of mammals. Thus, the hepatic lobule structures were more complete than those seen in Otocephala and Elopomorpha.

Sinusoidal structures: The sinusoidal structures were en-

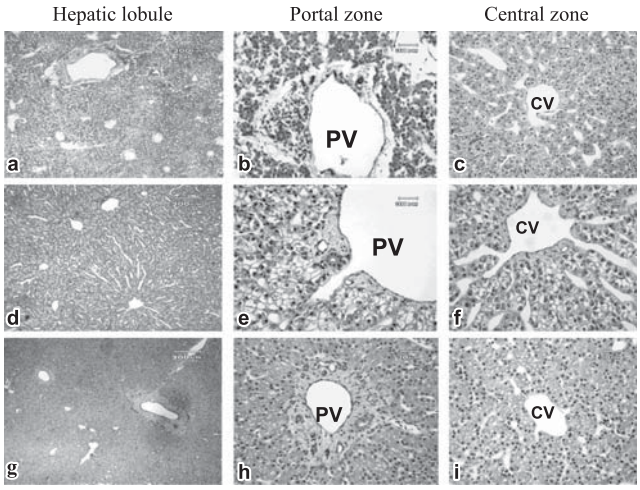

Fig. 1 : Low and high magnification of light micrographs of livers of the infradivision– Elopomorpha. The liver is mainly composed of a compact field of hepatocytes. *Anguilla japonica* (a–c); *Gymnothorax pictus* (d–f); *Conger japonicus* (g–i). The Glisson's sheath (portal vein, hepatic artery and bile duct) and the sinusoidal structures were the main structural unit of the liver lobule. The intrahepatic plates were tubular form with several cell thick of hepatocytes radiating from the portal veins to the central veins and the sinusoidal capillaries were narrow and irregularly shaped appearing throughout the interstice between the hepatic plates clearly visible in the highly magnified micrographs.

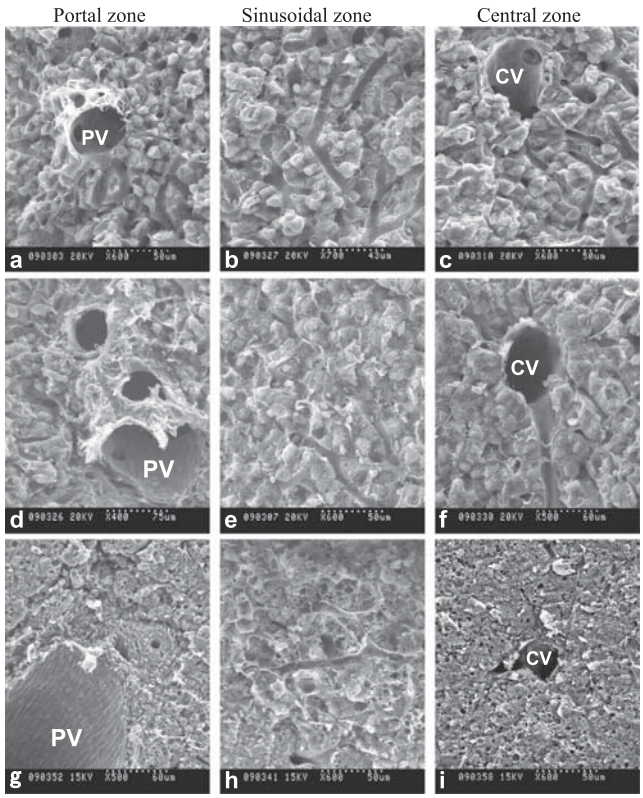

Fig. 2 : Low and high magnification of scanning electron microscopic (SEM) photographs of liver of the infradivision–Elopomorpha, illustrating the microanatomy of hepatic lobule. Sinusoidal structures are visible in all micrographs. *Anguilla japonica* (a–c); *Gymnothorax pictus* (d–f); *Conger japonicus* (g–i). CV: Central venule, PV: Portal venule.

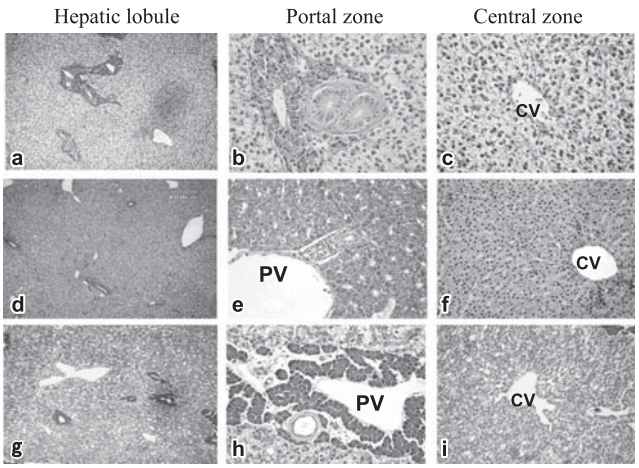

Fig. 3：Low and high magnification of light micrographs of livers of the infradivision–Otocephala. The hepatic lobule composed of a continuous compact field of connective tissue surrounded among hepatocytes. The intrahepatic arrangements were tubular form distributed perpendicularly from portal veins to the central veins and the cytoplasm of hepatocytes contains glycogen, *Ischikauia steenackeri* (d–f). The sinusoidal structures were narrow tubular in shape surrounded with two layers of polyhedral nucleated hepatocytes. The nuclei were usually small and located near the central area. *Cyprinus carpio* (a–c).

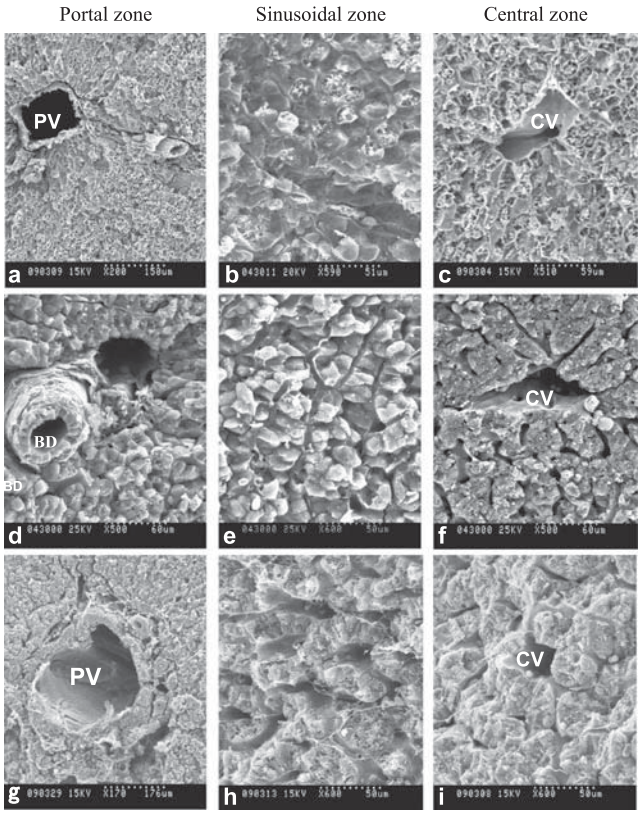

Fig. 4：Low and high magnification scanning electron micrographs of liver of the infradivision– Otocephala. Two layers of hepatocytes lining were visible between the sinusoids in the liver lobule, *Ischikauia steenackeri* (d–f). The hepatocytes were polygonal shape and the sinusoids were tubular form radiating from the central vein to the portal vein in *Cyprinus carpio* (a–c). The bile duct was accompanied with portal vein in *Plotosus lineatus* (g–i). CV: Central venule, PV: Portal venule, BD: Bile duct.

larged with more or less straight capillaries in *G. punctata* (Fig. 6h) and *L. japonicus* (Fig. 6a–c). In *P. trilineatum,* these structures were compactly arranged with two layers of hepatocytes (Fig. 5e).

Interrelation between parenchymal arrangements and phylogenic branching

Our analysis of these fish groups revealed a clear interrelation between hepatic parenchymal structures and phylogeny (Fig. 7). The parenchymal arrangements seemed to parallel the phylogenic advancement. As phylogenic branching is graded in ascending order from the primary to the advanced level, the parenchymal arrangements progressed from the solid or tubular to cord–like. Nuclear positioning is also very important for liver ontogenesis. Hepatocytes with centrally located nuclei have higher physiological function, and the position of the nuclei shifts from the periphery to the center of hepatocytes from Elopomorpha to Euteleostei.

Discussion

The present report describes the hepatic parenchymal architecture of nine teleost taxa, belonging to three infradivisions: Elopomorpha, Otocephala, and Euteleostei. In Elopomorpha, the hepatic lobule structures were incomplete; there were several layers of hepatocytes with tubular intra–hepatic capillaries. The layers, two cells thick, were observed in the Otocephala though the intra–hepatic capillaries were tubular. In addition, the cytoplasm of the nucleated hepatocytes contained glycogen (Gonzalez et al. 1993). In Euteleostei, the hepatic lobular structures were more advanced than those of other fish groups and more similar to those of the mammalian liver, where the lobular structures were cord–like and the intra–hepatic structures were compactly arranged with two layers of hepatocytes.

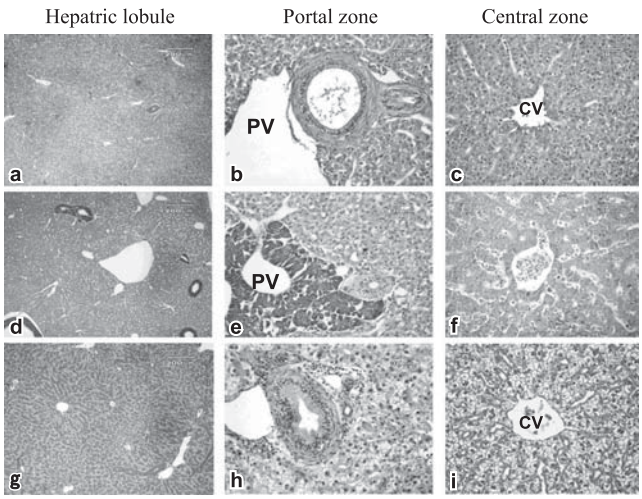

Fig. 5 : Low and high magnification of light micrographs of livers of the Infradivision- Euteleostei. The hepatic lobular arrangements were two cell thick plate type and the bile ducts were accompanied with a portal venule and hepaic arteriole similar to mammalian portal tructs, *Parapristipoma trilineatum, Girella punctata* (d-f; g-i). The intrahepatic structures were cord like form in both *Girella punctata* and *Parapristipoma trilineatum* and hepatocytes lining is simple layered, polyhedral shape with a round nucleus. The sinusoidal structures were enlarged with straight capillaries (*Girella punctata*), and this structures were compactly arranged with two layers of hepatocyte radiating from the central venule to the portal venule, *Lateolabrax japonicus* (a-c).

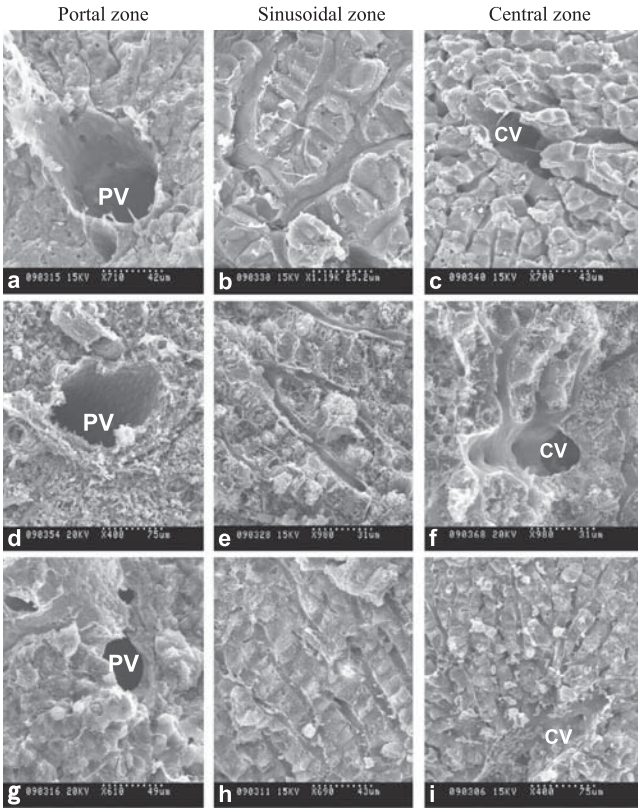

Fig. 6 : Low and high magnification of scanning electron micrographs of livers of the infradivision- Euteleostei. The hepatic lobular arrangements were two cell thick plate type and the bile ducts were accompanied with a portal venule and hepaic arteriole similar to mammalian portal tructs, *Girella punctata* (g-i). The intrahepatic structures were cord like form in both *Girella punctata* and *Parapristipoma trilineatum* and hepatocytes lining is simple layered, polyhedral shape with a rounded nucleus. The sinusoidal structures were enlarged with straight capillaries (*Girella punctata*), and this structures were compactly arranged with two layers of hepatocyte radiating from the central venule to the portal venule, *Lateolabrax japonicus* (a-c).

This study is to investigate the hepatic parenchymal structure using a scanning electron microscope. We aimed to describe the structural units of hepatic parenchyma, such as the Glisson's sheath, portal and central veins, and the biliary network, to evaluate structural differences in the context of phylogeny relationships. Hepatology of vertebrates is largely based on studies of mammalian livers, especially those of rodents and humans. Although less known, and less studied, fish livers are of great interest. Indeed, given that there are approximately 20,000 to 25,000 fish species, the description of any specific liver can hardly be used as a standard model for the Teleostei, although common inter-order morphologic features have been determined. In addition to this inter-specific variability, some physiological characters of fish contribute to amplify their hepatic polymorphism. The fish liver plays an important role in vitellogenesis and, when compared with mammals, only a minor role in carbohydrate metabolism. In contrast, the fish liver should be considered a target organ of many biological and environmental parameters, including food, pollutants, toxins, parasites, and microorganisms, that can alter liver structure and metabolism (Brusle and Anadon 1996).

A major issue in the liver histology of fish that have no pancreas is the correct identification of all the afferent and efferent veins. This problem is significant because many of the isolated veins observed in sections prove to be afferent and because of the aforementioned fact that spatial arrangements of biliary, arterial, and venous distributions always begin to differ at some point within the liver; a phenomenon that can be seen in the human liver pathologies. However, and under normal circumstances, in mammals,

structural associations (portal triads) between ramification of the portal veins, hepatic arterioles, and biliary channels facilitate recognition of afferent and efferent veins and zones of the hepatic lobules, irrespective of their diverse types and shapes. In livers of fish that harbor exocrine pancreocytes, the presence of such cells in the adventitia of veins has long been considered a sufficient criterion to accurately identify the portal venous branches (Figueiredo-Fernandes et al. 2007).

Many reports have been published on the nuclear deviation in the hepatic parenchymal cells on the sinusoidal surfaces (Sato et al. 2001). In normal rat and human livers, most of the nuclei of hepatic parenchymal cells are centrally located in the cytoplasm. However, it has been reported that the nuclei of hepatic parenchymal cells deviate position on sinusoidal surfaces during regeneration and under several pathological conditions, including chronic hepatitis, hepatocellular carcinoma, and adenomatous hyperplasia (Sato et al. 2001). In contrast to the situation in mammals, a significantly higher frequency of nuclear deviation in hepatic parenchymal cells on the sinusoidal surfaces was seen in the teleost fish. We observed deviated nuclear positioning in nine species belonging to the three infradivision of teleost. In Elopomorpha, the nuclei were observed at the periphery of the hepatocyte cytoplasm near the sinusoidal surfaces. On the contrary, in the three species of Euteleostei, the nuclei were both in the center and on the sinusoidal surface. The polarity of the hepatic parenchymal cells, important for the proper physiological functioning of the liver, appears to be regulated by cell-cell and cell-extracellular matrix interactions, and is associated with a reorganization of the cytoskeleton and organelles, including nuclei, Golgi complexes, and mitochondria.

The essential feature of the hepatic parenchyma is lobulation. The teleost livers showed a great diversity of structure in both light and scanning electron micrographs. The hepatic lobules of fish could be classified into three distinct types, cord-like (two cell), tubular (two to several cells), and solid (several cells arrangements). It is known that the parenchymal arrangements in normal humans are one cell thick, but the parenchyma in livers of other fish groups are two to several cells thick. In this study, some fish livers had a more or less similar structure to normal human livers while others were modified and appeared to be a more primitive type; for example, the cord-like form was observed in Euteleostei, and the solid and tubular forms were recognized in both Elopomorpha and Otocephala. This study showed that Euteleostei is the most recent phylogenetic branch among Teleostei, followed by Otocephala and Elopomorpha with secondary and primarily branches, respectively. In addition, the parenchymal arrangements progressed from solid or tubular to cord-like, while the shape of hepatocytes changed from round to square or polyhedral. In the cord-like parenchyma, hepatocytes were in close contact with the sinusoidal capillaries that form a dense network, as in mammalian livers.

Conclusion

1. Fish liver in species from the most recent phylogenic branch (Euteleostei) had an advanced hepatic lobule structure similar to the arrangement in mammals, which possess higher metabolic function.
2. Fish liver from species within the primary or secondary phylogenic branches (Elopomorpha and Otocephala, respectively) had sinusoids of a more primitive form that were narrow with an undeveloped hepatic lobular network, similar to those of lower vertebrates.
3. The parenchymal arrangements evolved with phylogenic advancement, and these structural changes reflect the route of hepatic ontogenesis.
4. The connective tissue layer around the portal vein and central vein gradually complete/develop from Elopomorpha to Euteleostei.
5. Nuclear positioning gradually shifted from the periphery to the center of hepatocytes, and the more centrally located nuclei indicated higher metabolic function.

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