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Source: Zoological Science, 21(8) : 841-850

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.21.841

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ZOOLOGICAL SCIENCE **21**: 841–850 (2004)
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Comparative Histological Study of Teleost Livers in Relation to Phylogeny

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ABSTRACT—This report presents a detailed description of hepatic architecture in 200 teleost livers by light microscopy and extensively discusses the phylogenetic viewpoint. The 200 teleost livers showed a great variety of histological images, but not the same image, as in mammalian livers. The hepatocyte-sinusoidal structures of the fish livers were classified into three different types: (a) cord-like form, (b) tubular form, (c) solid form. Biliary tract structures were classified into four types: (a) isolated type, (b) biliary-arteriolar tract (BAT) type, (c) biliary-venous tract (BVT) type, and (d) portal tract type. As phylogenic advancement is graded from low to high, the parenchymal arrangement progressed from solid or tubular form to cord-like form, but the biliary tract structures were not involved. We demonstrate that this study is the first to investigate teleost livers phylogenically, and their architectural differences are shown in the route of hepatic ontogenesis. In hepatic ontogenesis, the formation of the parenchymal arrangement is acquired phylogenically, but the biliary pathway may be adapted in the ecological and behavioral patterns.

Key words: liver, teleost, phylogeny, morphology, evolution

INTRODUCTION

The liver is the largest internal organ of the body and the largest gland tissue. It is the organ in which nutrients absorbed in the digestive tract are processed and stored for use by other parts of the body. The metabolism has various functions (e.g. protein synthesis, storage metabolites, bile secretion, detoxification and inactivation) that play a central role into maintaining life. The liver receives blood through both the portal vein and hepatic artery. Most of its blood (70–80%) comes from the portal vein that conveys blood containing nutrients absorbed in the intestine. The hepatic artery, a branch of the celiac axis, is oxygenated in the liver (Rappaport, 1963).

The structural and functional unit of the liver is the acinus, in which are both the hepatic lobule and portal triad (also called Glisson's sheath or portal tracts). The hepatic lobule consists of hepatocytes that are the functional center of the liver, and in which the hepatocyte-sinusoidal structures are formed. The sinusoids are capillary networks, and are localized in the space between hepatic plates in which the hepatocytes are arranged (Rappaport, 1963; Motta, 1984). In mammals and higher vertebrate animals, hepatic plates line the simple-layered hepatocytes, so-called one-cell-thick plates, and pass through from the portal triad to the central vein located in the center of the hepatic lobule (Elias and Bengelsdorf, 1952). The portal triad is located in the portal spaces between the hepatic lobules, and contains branches of the portal vein, hepatic artery, and bile duct, lymph vessels and nerves. These vessels and ducts are surrounded by connective tissue (Motta, 1984) .

In teleost livers, a large number of morphological studies of the hepatic cells, hepatocytes (Langer, 1979; Nopanitaya *et al*., 1979b), endothelial cells (Ferri and Sesso, 1981; Sato and Yamamoto, 1983), hepatic stellate cells (Nopanitaya *et al*., 1979b; Eastman and De Vries, 1981), Kupffer cells (Hampton *et al*., 1987; Rocha *et al*., 1997) and bile ductules cells (Shin, 1978; Tanuma, 1980; Satoh, 1983), in the hepatic lobule, and the biliary system (Hampton *et al*., 1985; 1988) and innervation (Esteban *et al*., 1998), have revealed details of their connection (Sakano and Fujita, 1982; Hampton *et al*., 1989; Speilberg *et al*., 1994), and established the liver structures and functions (Motta, 1984). The recent aims have been as follows: (1) ecological and toxicological studies of the liver as a biomarker of environmental pollution (Braunbeck, 1994), (2) a histochemical study to establish whether the liver has a central role in metabolism (Orbea *et al*., 1999; Jung *et al*., 2002), (3) a pathological study of the liver as an important organ for the analysis of fish diseases (e.g., cholestasis and neoplasm)(Couch, 1991; 1993; Okihiro and Hinton, 2000). From

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current zoological viewpoints, the themes of biodiversity or evolution have been focused and investigated (Gemballa *et al*., 2003), but there has been little phylogenic research (Satoh, 1983; Cornelius, 1985; Akiyoshi *et al*., 2001) in any vertebrates into the evolution of the liver.

Although teleost fish number approximately 25,000 species, the phylogenic grade is clearly categorized (Nakabo, 2000). Thus, a phylogenic study of fish livers may be valid as an optimal model for liver ontogenesis in vertebrates. To demonstrate the correlation between the liver structures and phylogenic status, we observed 200 teleost livers by light microscope, and subjected the data to phylogenic analyses. We focused on the architecture of the hepatocyte-sinusoidal arrangements and the biliary tract structures.

MATERIALS AND METHODS

Sample collection

For this comparative morphological study, the livers of 200 different teleost species were used (Table 1). We collected 48 fish species from rivers and two lakes, the Nakaumi and the Shinjiko in Shimane Pref., 98 species from the coast of Shimane Peninsula and the Oki Islands in Shimane Pref., and 54 species from the coast and river mouth of Iriomote Island in Okinawa Pref. In order to eliminate the influence of seasonal changes or growth, all specimens were caught in the adult or semi-adult stage from April to October, and three to five specimens were sampled respectively. All fish were caught with traps and hand nets in each locality from 2000 to 2003. The phylogenic status in Class Osteichthyes, comprising three infradivisions of Teleostei: 6 Elopomorpha, 30 Otocephala, and 164 Euteleostei species, is shown in Table 2.

Histology and histochemistry for neutral lipids

The livers were perfusion-fixed via the portal vein with 4% paraformaldehyde buffered at pH 7.4 with 0.1 M phosphate for 15 min, cut into small pieces, and immersed in the same solution for 3 days at 4°C. The specimens were rinsed, dehydrated and embedded in paraffin. Serial 4 μm sections were obtained, and some of these were stained with both hematoxylin and eosin.

The histochemical demonstration of neutral lipids in the hepatocytes was performed according to the method of Oil-red-O staining. Briefly, the livers were sectioned into 30 μm slices with a Dosaka microslicer and rinsed with 0.1 M phosphate buffer (pH 7.4). The sections were washed thoroughly with distilled water and 60% isopropyl alcohol, followed by incubation with a filtered 0.3% working solution of Oil red-O (Wako Pure Chemical, Osaka), and heated to 37°C for 12 min. The sections were then stained with hematoxylin.

RESULTS

The results of hematoxylin and eosin staining for hepatocyte-sinusoidal structures and biliary tract structures in the livers of 200 fish are summarized in Table 1. The 200 teleost livers showed great variety in the microscopic images, but not the same image as is seen in mammalian livers. In almost all fish, the structural units known as hepatic lobules were absent from connective tissue septa. The liver was mainly composed of a continuous compact field of hepatocytes, and scattered with islands of connective tissue enclosing the bile duct and arterial vessels (Fig. 1a). In a few, the hepatic lobules were demarcated by connective tissue containing bile ducts, portal and arterial vessels similar to portal tracts in mammals (Fig. 1b).

Parenchymal arrangement (hepatocyte-sinusoidal structures)

Following portal venous perfusion fixation, hepatic sinusoids were cleared of blood cells and the definition of hepatocyte-sinusoidal structures was enhanced. The hepatocyte-sinusoidal structures of fish livers were classified into three different types: (a) cord-like form, (b) tubular form, and (c) solid form. In the cord-like form (Fig. 2a), the majority of the hepatocyte lining was simple-layered. The hepatic sinusoids were enlarged with straight capillaries connecting through the perilobular to the centrolobular vessels. The hepatocytes were polyhedral, and had a rounded nucleus. In the tubular

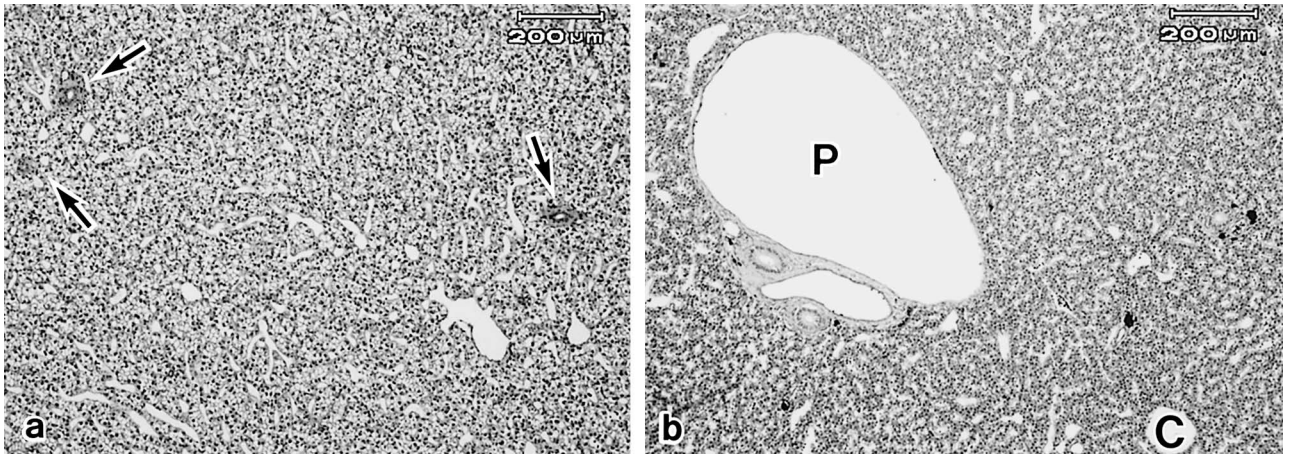


Fig. 1. Low magnification light micrographs of hepatic lobule in livers. a) The liver is mainly composed of a continuous compact field of hepatocytes, and scatter with islands (arrows) of connective tissue enclosing the bile duct and arterial vessels. The hepatic lobule is absent from connective tissue septa. *Gymnothorax kidako*. b) The portal triad (P) is seen the portal spaces in hepatic lobule, and contains branches of the portal vein, hepatic artery and bile ducts. *Oncorhynchus mykiss*. C: central vein

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form (Fig. 2b), the majority of the hepatocyte lining was double-layered. The sinusoidal capillaries were narrow and irregularly shaped sinusoids appearing throughout the interstice between the hepatic plates. Three to four hepatocytes surrounded a sinusoidal capillary. The hepatocytes were polyhedral or rounded, and had a rounded nucleus. In the solid form (Fig. 2c–f), the major part of the hepatocyte lining was multi-layered. The hepatic sinusoids were narrow and

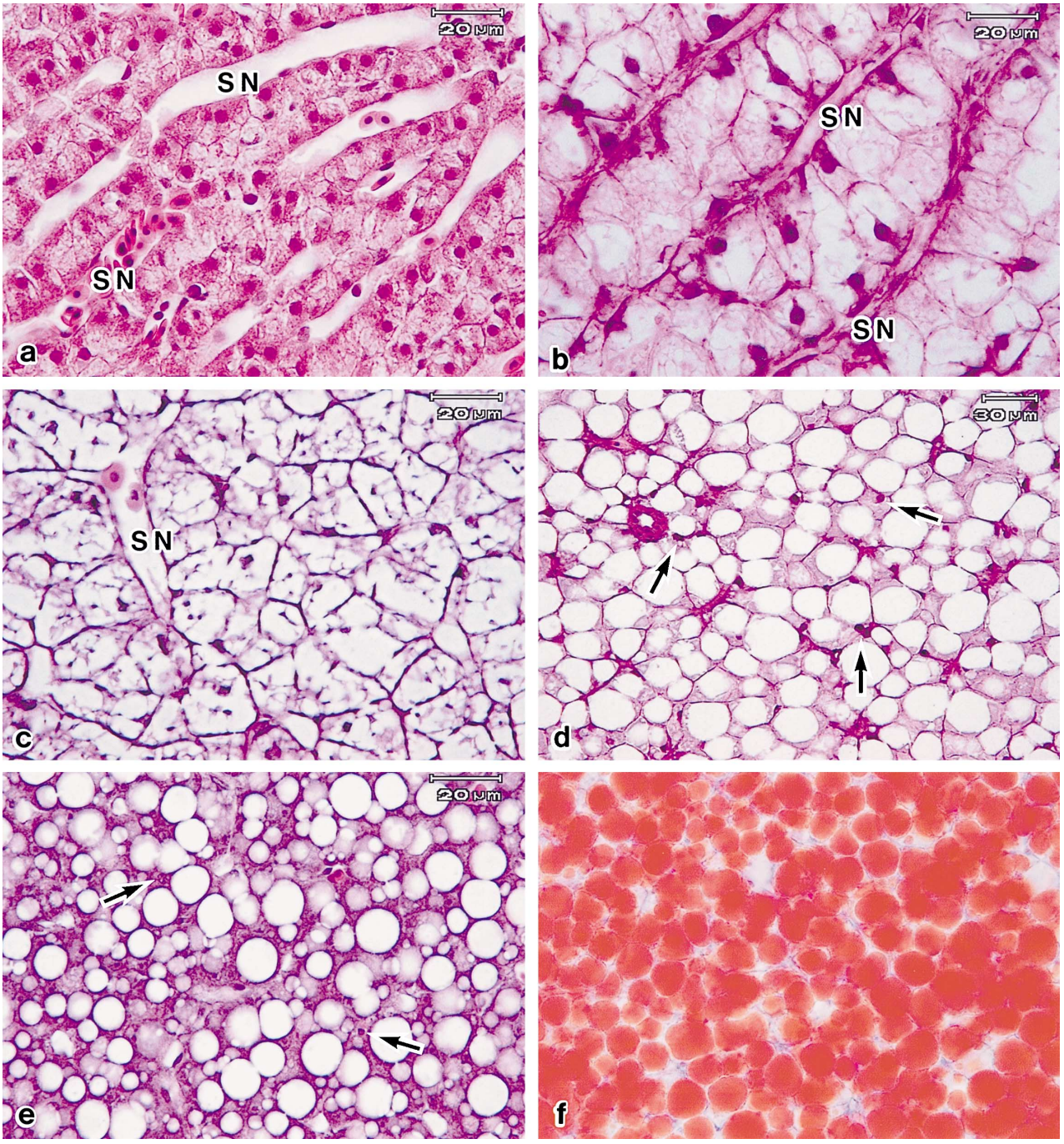


Fig. 2. High magnification light micrographs of hepatocyte-sinusoidal structures in livers. a) Cord-like form. The hepatocyte lining is simple-layered. The hepatic sinusoids (SN) are enlarged with straight capillaries. The hepatocytes are polyhedral, and have a rounded nucleus. *Girella punctata*. (b) Tubular form. The hepatocyte lining is double-layered. The sinusoidal capillaries (SN) are narrow and irregularly shaped sinusoids appearing throughout the interstice between the hepatic plates. The hepatocytes are polyhedral or rounded, and have a rounded nucleus. *Pterois lunulata*. c) Solid form. The hepatocyte lining is multi-layered. The hepatic sinusoids (SN) are narrow and short tortuous capillaries. *Inimicus japonicus*. d) In Gobioidei, the cytoplasm of the hepatocytes is filled with fat droplets. The hepatocytes are rounded, and have a rounded small nucleus (arrows). *Gymnogobius castaneus*. e) In Tetraodontiformes, the numerous fat droplets are observed in the hepatocytes. The hepatocytes are rounded, and few small nuclei (arrows) are seen among the fat droplets. *Takifugu poecilonotus*. f) These fat droplets are stained with red color that is neutral lipids. Oil red-O staining. *Takifugu poecilonotus*.

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Table 1. Summary of the expression levels of hepatocyte-sinusoidal structures and biliary tract structures in 200 teleost livers.

Species	Sinusoid	Biliary Tract
Elopomorpha		
Anguilliformes		
Anguilla japonica	2	–
Gymnothorax kidako	2	1・2
Ophisurus macrorhynchus	2	–
Conger myriaster	2	2・4
Conger japonicus	2	2・4
Muraenesox cinereus	2	2・4
Otocephala		
Clupeiformes		
Etrumeus teres	1	–
Sardinella zunasi	2	1
Konosirus punctatus	2	1
Engraulis japonicus	2	1・3
Cypriniformes		
Cyprinus carpio	2	1・2
Carassius auratus langsdorfii	2	1・2
Carassius auratus	2	1・3
Tanakia lanceolata	1	1
Tanakia limbata	2	1・2
Acheilognathus rhombeus	2	1・2
Rhodeus ocellatus ocellatus	2	1
Ischikauia steenackeri	2	1・2・4
Zacco platypus	2	1・2・4
Zacco temminckii	2	1・2
Phoxinus lagowskii steindachneri	2	2
Phoxinus oxycephalus jouyi	2	1・2
Tribolodon hakonensis	2	1・2・4
Pungtungia herzi	2	1・2
Gnathopogon caerulescens	1	1・2
Pseudogobio esocinus esocinus	1	1・2
Hemibarbus longirostris	1	1・2
Squalidus gracilis gracilis	2	1・2・4
Squalidus chankaensis biwae	1	1
Cobitis biwae	2	1・4
Cobitis sp. 2 subsp. 3	2	1
Lefua sp.	2	1
Siluriformes		
Pseudobagrus nudiceps	2	–
Silurus asotus	2	1・3
Liobagrus reini	2	–
Plotosus lineatus	2	1・3
Euteleostei		
Salmoniformes		
Hypomesus nipponensis	2	–
Plecoglossus altivelis altivelis	2	1
Salangichthys microdon	2	–
Salvelinus leucomaenis pluvius	2	1・2・4
Salvelinus leucomaenis imbrius	2	1・2・4
Oncorhynchus mykiss	3	1・4
Oncorhynchus keta	2	1・4
Oncorhynchus masou masou	2	1・4
Oncorhynchus masou ishikawae	2	1・2
Beryciformes		
Sargocentron microstoma	2	1・2・3
Myripristis berndti	2	1・3
Monocentris japonica	2	1・3
Gasterosteiformes		
Gasterosteus aculeatus	2	1
Fistularia petimba	2	1
Fistularia commersonii	2	–
Microphis brachyurus brachyurus	2	–
Mugiliformes		
Mugil cephalus cephalus	2	1・4
Chelon haematocheilus	2	1・3・4
Hypoatherina valenciennei	2	1
Oryzias latipes	2	1・3
Hyporhamphus sajori	2	1・4
Cypselurus agoo agoo	2	1・4
Scorpaeniformes		
Pterois lunulata	2	1・2・4
Sebastiscus marmoratus	2	2・4
Sebastes inermis	2	1・2・4
Sebastes schlegelii	2	1・4
Sebastes pachycephalus pachycephalus	2	2・4
Inimicus japonicus	1	1・2・4
Hypodytes rubripinnis	2	1・4
Chelidonichthys spinosus	2	2
Platycephalus sp. 2	1	1・2
Cociella crocodila	2	1・2
Pleurogrammus azonus	1	1・2・4
Hexagrammos agrammus	2	2
Hexagrammos otakii	2	1・3
Cottus kazika	1	–
Cottus nozawae	1	1
Pseudoblennius cottoides	2	1・2
Pseudoblennius percoides	2	1・2
Perciformes		
Percoidei		
Coreoperca kawamebari	2	1・2
Lateolabrax latus	2	1・2・4
Lateolabrax japonicus	2	1・2・4
Cephalopholis urodeta	3	1・2・3
Epinephelus akaara	3	1・2・3
Epinephelus fasciatus	3	1・2・3
Epinephelus merra	2	1・2・3
Lepomis macrochirus	2	2
Micropterus salmoides	3	1・2・4
Heteropriacanthus cruentatus	2	1・2・4
Apogon notatus	2	1・3
Scombrops gilberti	2	1・2
Coryphaena hippurus	3	1・2・4
Seriola dumerili	3	1・2
Trachurus japonicus	3	1・4
Selar crumenophthalmus	3	1・4
Caranx melampygus	3	1・2・4
Gnathanodon speciosus	3	1・4
Leiognathus nuchalis	3	2
Lutjanus argentimaculatus	3	1・2
Lutjanus decussatus	3	1・2・4
Lutjanus fulvus	3	2
Hapalogenys nigripinnis	3	1・4

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Species	Sinusoid	Biliary Tract
Parapristipoma trilineatum	3	2・3
Plectorhinchus cinctus	3	1・4
Pentapodus caninus	2	1・3
Acanthopagrus schlegelii	3	1・3
Acanthopagrus berda	3	1・3
Pagrus major	3	1・3
Dentex tumifrons	3	1・3
Gnathodentex aureolineatus	2	2・3
Lethrinus genivittatus	2	2
Sillago japonica	3	2
Sillago parvisquamis	3	2・4
Upeneus tragula	3	1・3
Parupeneus multifasciatus	3	2・4
Parupeneus indicus	3	1・3・4
Oreochromis mossambicus	3	1・2・4
Ditrema temmincki	3	1・2・3
Chromis notata miyakeensis	3	1・3
Dascyllus trimaculatus	3	1・3
Abudefduf bengalensis	3	1・3
Amblyglyphidodon curacao	3	1・3
Terapon jarbua	3	1・2・4
Rhyncopelates oxyrhynchus	2	2・4
Oplegnathus fasciatus	2	1・2
Oplegnathus punctatus	3	1・2
Kyphosus vaigiensis	3	1・2
Girella punctata	3	1・2・4
Labroidei		
Choerodon azurio	1	1・3
Semicossyphus reticulatus	2	1・4
Bodianus perditio	2	1・2
Pseudolabrus sieboldi	2	1・3
Halichoeres trimaculatus	2	1・2
Halichoeres poecilopterus	2	1・2
Epibulus insidiator	2	2
Zoarcoidei		
Zoarchias glaber	3	–
Dictyosoma burgeri	2	1・2・4
Pholis nebulosa	1	1・2
Trachinoidei		
Parapercis polyophthalma	3	1・2
Uranoscopus japonicus	1	1
Blennioidei		
Enneapterygius etheostomus	2	1
Neoclinus bryope	1	1
Parablennius yatabei	2	1
Omobranchus elegans	2	1
Petroscirtes breviceps	2	1
Gobioidei		
Odontobutis obscura	2	1・2・4
Eleotris fusca	2	1・2
Boleophthalmus pectinirostris	1	1・2
Periophthalmus modestus	1	1
Periophthalmus argentilineatus	1	1
Taenioides rubicundus	1	1
Sicyopterus japonicus	1	1
Stiphodon percnopterygionus	1	–
Leucopsarion petersii	2	–
Chaenogobius annularis	1	1・2
Chaenogobius gulosus	1	1・2
Gymnogobius petschiliensis	1	1・3
Gymnogobius urotaenia	1	1
Gymnogobius castaneus	1	1
Gymnogobius taranetzi	1	1
Glossogobius olivaceus	1	1・2
Yongeichthys criniger	2	1
Acanthogobius flavimanus	2	1・2・4
Acanthogobius lactipes	1	1
Exyrias puntang	1	1
Amblygobius phalaena	1	–
Mugilogobius abei	1	2
Acentrogobius pflaumii	1	1・2
Rhinogobius giurinus	1	1
Rhinogobius sp. DA	1	1
Rhinogobius sp. OR	1	1・2
Rhinogobius sp. YB	1	1・2
Rhinogobius flumineus	1	1・2
Tridentiger bifasciatus	1	1
Tridentiger brevispinis	1	1・2
Tridentiger obscurus	1	1・2
Acanthuroidei		
Siganus unimaculatus	3	1・2・4
Siganus fuscescens	2	1・2
Scombroidei		
Sphyraena barracuda	3	1
Sphyraena pinguis	3	1・3
Trichiurus japonicus	3	1・2・4
Katsuwonus pelamis	3	1・2
Scomberomorus niphonius	3	1・2・4
Pleuronectiformes		
Paralichthys olivaceus	2	2・4
Bothus mancus	2	1・4
Kareius bicoloratus	2	4
Zebrias zebra	2	1・2
Arelia bilineata	2	1・2
Paraplagusia japonica	2	1・2
Tetraodontiformes		
Balistoides conspicillum	1	4
Balistapus undulatus	1	1
Rhinecanthus aculeatus	1	1・2
Aluterus scriptus	1	1・4
Rudarius ercodes	1	1・3
Thamnaconus modestus	1	1
Stephanolepis cirrhifer	1	1・2
Ostracion immaculatus	1	2
Takifugu pardalis	1	1・2
Takifugu snyderi	1	1・2
Takifugu poecilonotus	1	1・2
Takifugu niphobles	1	1・2
Takifugu rubripes	1	1・2
Chelonodon patoca	1	1・2
Diodon holocanthus	1	1

Sinusoid: hepatocyte-sinusoidal structures (1):solid form, (2): tubular form, (3): cord-like form. Biliary Tract: (–):no bile duct, (1): isolated type, (2): biliary-arteriolar tract type, (3): biliary-venous tract type, (4): portal tract type.

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short tortuous capillaries. The hepatocytes were rounded, and had a rounded small nucleus. The cytoplasm of the hepatocytes was occasionally filled with fat droplets. In particular, in Gobioidei (Fig. 2d) and Tetraodontiformes (Fig. 2e), all species had numerous fat droplets in the hepatocytes. These fat droplets were confirmed as neutral lipids by the Oil red-O staining method (Fig. 2f).

In Elopomorpha, the parenchymal arrangement of Anguilliformes was of tubular form. Otocephala, Cluperiformes and Siluriformes also had a tubular form, but some Cypriniformes were of solid form. On the other hand, Euteleostei, Salmoniformes, Beryciformes, and Mugiliomorpha also had a tubular form, but the livers of Scorpaenoidei had both solid and tubular forms. The livers of Perciformes had a cord-like form, except the livers of Gobioidei. Pleuronectiformes had a tubular form. Almost all Gobioidei and Tetraodontiformes fish had a solid form, and the hepatocytes were filled with numerous fat droplets in the cytoplasm. Few nuclei were found among the fat droplets.

Biliary tract structures

Biliary tract structures were classified into four types according to whether the bile duct is accompanied with blood vessels: (a) isolated type, (b) biliary-arteriolar tract (BAT) type, (c) biliary-venous tract (BVT) type, and (d) portal tract type. In the isolated type (Fig. 3a), a bile duct was located independently in the hepatic lobules and was surrounded by connective tissue as a sheath. Ultimately, almost all intralobular bile ducts were amalgamated to form the bile duct in the portal tract, but some were completely isolated without blood vessels. In the BAT type (Fig. 3b), bile ducts were accompanied with an arteriole and were observed in the hepatic lobule. These ducts contained no venous profiles, and were observed in the connective tissue sheath surrounding BAT. The BAT type was observed in almost all species, and had two pathways that combined with either the isolated type or the portal-tract type. In the BVT type (Fig. 3c), a bile duct was accompanied with a portal venule and was located in the hepatic lobule. The BVT type was also surrounded by connective tissue as a sheath, although this type was very rare. In the portal-tract type (Fig. 3d), the bile duct was accompanied by a portal venule and hepatic arteriole as in mammalian portal tracts. This type was widely observed in many species, and was combined with either the isolated type or BAT type.

In Elopomorpha, the biliary tract structures of Anguilliformes were shown in the portal-tract type, and some combined the isolated or BAT type. Otocephala, Cluperiformes, Cypriniformes and Siluriformes had the isolated type, but many Cypriniformes combined the BAT and/or portal-tract type. In Euteleostei, almost all species also had the isolated type, and most fish, except Gobioidei and Tetraodontiformes, combined the BAT and/or portal-tract type as in Otocephala. On the other hand, in Gobioidei and Tetraodontiformes, the biliary tract structures were of the isolated type and/or BAT type, but portal tracts were not formed.

Table 2. Summary of the phylogenic status in class Osteichthyes.

Class Osteichthyes	
Subclass Actinopterygii	
Division Neopterygii	
Subdivision Teleostei	
Infradivision Elopomorpha	
Order Anguilliformes	6 species
Infradivision Otocephala	
Order Clupeiformes	4 species
Order Cypriniformes	22 species
Order Siluriformes	4 species
Infradivision Euteleostei	
Order Salmoniformes	9 species
Order Beryciformes	3 species
Order Gasterosteiformes	4 species
Order Mugiliformes	6 species
Order Scorpaeniformes	17 species
Order Perciformes	
Suborder Percoidei	49 species
Suborder Labroidei	7 species
Suborder Zoarcoidei	3 species
Suborder Trachinoidei	2 species
Suborder Blennioidei	5 species
Suborder Gobioidei	31 species
Suborder Acanthuroidei	2 species
Suborder Scombroidei	5 species
Order Pleuronectiformes	6 species
Order Tetraodontiformes	15 species

Interaction with the parenchymal arrangement and phylogeny

The correlation with hepatocyte-sinusoidal structures and phylogenic status is shown in Fig. 4. It seemed that the development of parenchymal arrangement was parallel to the phylogenic advancement. As phylogenic advancement is graded from low to high, the parenchymal arrangement progressed from the solid or tubular form to the cord-like form. Although Gobioidei, Pleuronectiformes and Tetraodontiformes have the highest phylogenic status among teleost fish, their parenchymal arrangement had solid and tubular forms.

Interaction with biliary tract structures and phylogeny

Throughout the class Osteichthyes, the biliary tract structures of almost all fish were the isolated type combined with either the BAT or portal tract type. The type of biliary tract structure varied, even in species of the same order, and it seemed that the structures were not related to phylogenic development.

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DISCUSSION

This study has shown that the hepatocyte-sinusoidal structures of the liver can be classified into three different types: (a) cord-like form (one-cell-thick plate type), (b) tubular form (two-cell-thick plate type), and (c) solid form (several cell-thick plate type). The classification was based on the investigation of Elias and Bengelsdorf (1952) in several mammals. It is well known that the parenchymal arrangement of normal humans is formed of a one-cell-thick plate, but the livers of lower vertebrates are two-cell-thick plates or several cell-thick plates (Elias and Bengelsdorf, 1952; Rappaport, 1963; Motta, 1984; Cornelius, 1985). In fish livers, previous studies described that some fish had a similar structure to normal humans, while others were modified in a more primitive form (Nopanitaya *et al*., 1979b; Speilberg *et al*., 1994; Akiyoshi *et al*., 2001). Olsson (1968) classified the hepatocyte arrangement into three different types: (a) compact type without cavities (advanced), (b) follicle type (intermediate), and (c) duct type (primitive). However, the structure of the primitive form is controversial (Langer, 1979; Hampton *et al*., 1985; MuCuskey *et al*., 1986; Biagianti-Risbourg, 1991; Speilberg *et al*., 1994), and several reports showed a branched-tubular form in some fish (Hinton *et al*., 1972; Nopanitaya *et al*., 1979b; Chapman, 1981; Braunbeck *et al*., 1987; Speilberg *et al*., 1994). Our 200 species study showed that the primitive form was a solid or tubular form, however, it is necessary to clarify the structure of the primitive form phylogenically.

This study is the first to investigate teleost livers phylogenically. We aimed to identify the interrelation of hepatocytes, sinusoids, and the biliary tract, and make a comparison with the phylogenic development. Mishra *et al*. (1988) revealed that differences in dietary habits had no bearing on the hepatic architecture in teleost livers. As the hepatic architecture is universal, we examined the correlation between hepatic architecture and phylogenic advancement.

The cord-like form was observed in Perciformes belonging to Euteleostei, and the primitive form was recognized in both Elopomorpha and Otocephala. It is well known that the phylogenic status in class Osteichthyes is clearly categorized (Table 2). Teleostei is classified into three

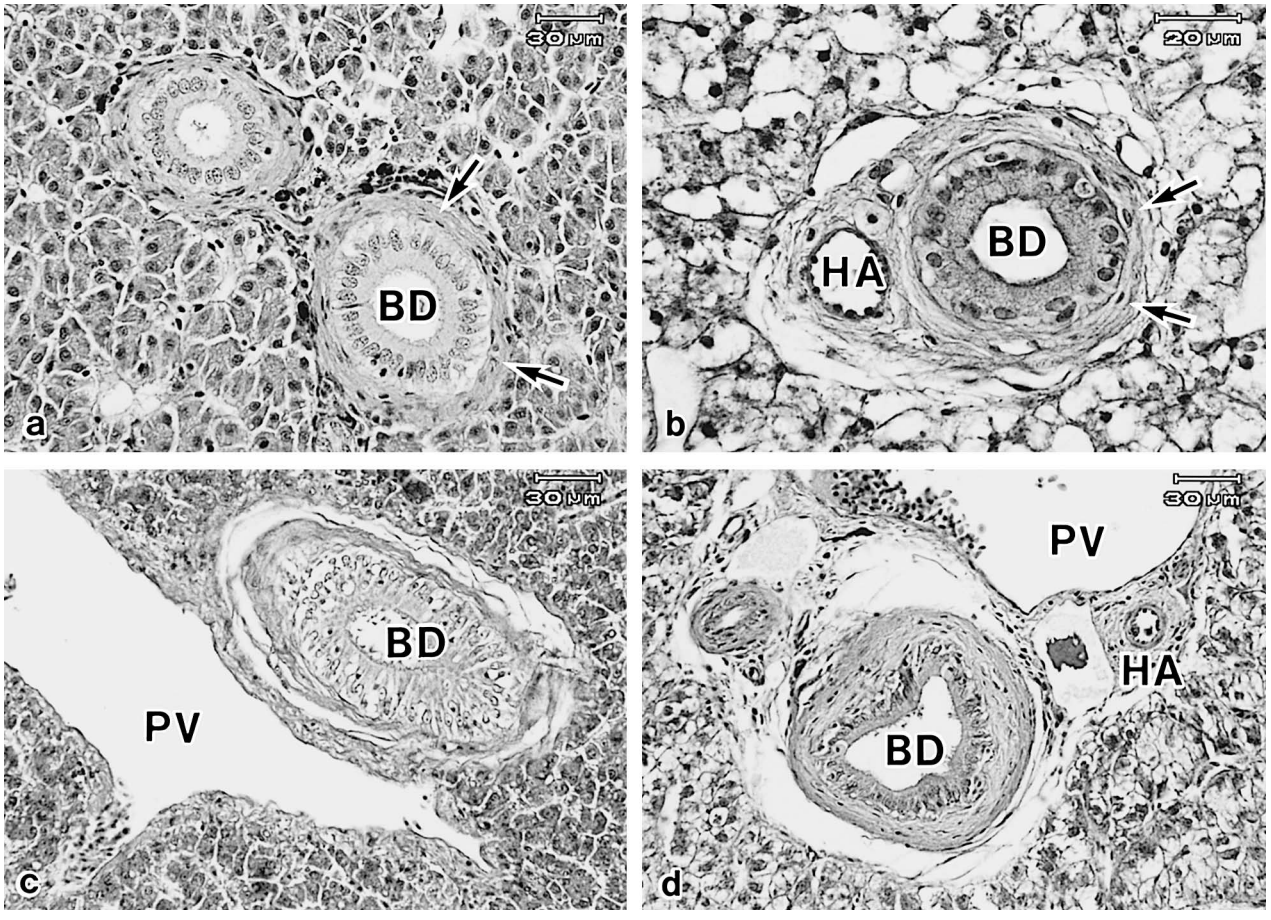


Fig. 3. High magnification light micrographs of biliary tracts structures in livers. a) Isolated type. A bile duct (BD) is located independently in hepatic lobules and is surrounded by connective tissue as a sheath (arrows). *Oncorhynchus masou masou*. b) Biliary-arteriolar tracts (BAT) type. A bile duct (BD) is accompanied with a hepatic arteriole (HA) in hepatic lobule. These ducts are observed in the connective tissue sheath (arrows) surrounding BAT. *Inimicus japonicus*. c) Biliary-venous tract (BVT) type. A bile duct (BD) is accompanied with a portal venule (PV) in hepatic lobule. The BVT type is also surrounded by connective tissue as a sheath. *Chelon haematocheilus*. d) Portal-tract type. The bile duct is accompanied by a portal venule (PV) and hepatic arteriole (HA) as in mammalian portal tracts. *Lateolabrax latus*.

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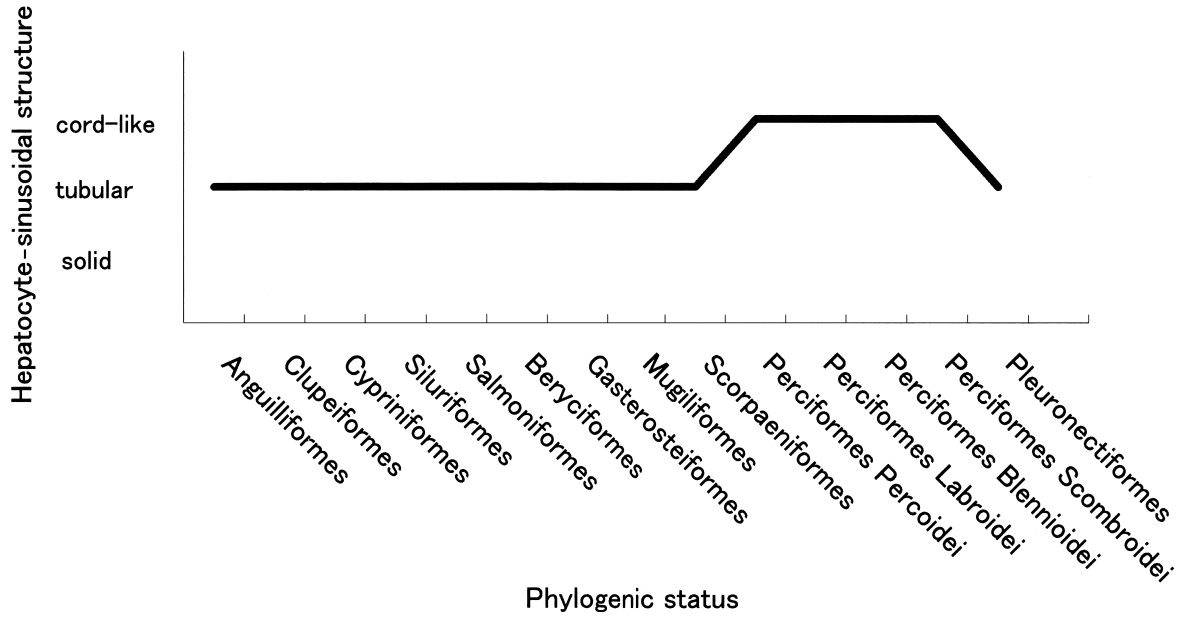


Fig. 4. Interaction with the parenchymal arrangement (hepatocyte-sinusoidal structures) and phylogeny. As phylogenic advancement is graded from low to high, the hepatocyte-sinusoidal structures progress from the tubular form to the cord-like form. Gobioidei in Perciformes and Tetraodontiformes are excepted.

infradivisions: Elopomorpha, Otocephala, and Euteleostei. Euteleostei has the highest phylogenic status among Teleostei, followed by Otocephala and Elopomorpha with the lowest status. This study showed that the parenchymal arrangement developed in parallel with phylogenic advancement. As phylogenic advancement is graded from low to high, the parenchymal arrangement progressed from solid or tubular to cord-like form, and the shape of hepatocytes changed from round to square and polyhedral cells.

In the circulatory system, the liver has an optimal position for gathering, transforming, and accumulating metabolites and eliminating substances. All materials are absorbed via the intestines, and reach the liver through the portal vein, except the complex lipids (chylomicrons), which are transported mainly by lymph vessels. Blood flows from the portal venules at the portal triads through the sinusoid and between the hepatic plates to the central vein (Rappaport, 1963). The hepatocyte-sinusoidal structure is physiologically important, not only because hepatocytes takes up large molecules from the sinusoid, but also because a large number of macromolecules (e.g., lipoproteins, albumin, fibrinogen) are secreted into the sinusoid. In the cord-like form, hepatocytes are closely contacted with sinusoidal capillaries that form a dense network as in mammalian livers (Elias and Bengelsdorf, 1952; Motta, 1984). In this study, we revealed that fish livers with a higher phylogenic status had structures identical to the mammalian arrangement, which possessed higher metabolic functions. In contrast, fish livers with a low phylogenic status had sinusoids of a primitive form, which were narrow with an undeveloped network, identical to lower vertebrates. We speculated that these structural changes reflect the route of hepatic ontogenesis, and are essential to the acquisition of higher hepatic function.

Bile is produced by hepatocytes and flows through the intrahepatocytic bile canaliculi, bile ductules in the hepatic lobule, and bile ducts in the portal tracts (Rappaport, 1963). Investigations into the biliary system of fish have mentioned intrahepatic canaliculo-ductular (C-D) junctions in which the Canal of Hering duct is located in the mammalian liver (Nopanitaya *et al*., 1979b; Tanuma, 1980; Hampton *et al*., 1985;1988). Comparative studies (Satoh, 1983) demonstrated that C-D junctions were classified into three types from fish to mammals. However, there are few reports in the comparative study of biliary tract structures in teleost livers (Sakano and Fujita, 1982; Satoh, 1983).

In this study, biliary tract structures were classified into four types: (a) isolated type, (b) biliary-arteriolar tract (BAT) type, (c) biliary-venous tract (BVT) type, and (d) portal-tract type. The BAT type was observed in almost all species, forming two passages, which combined with either the isolated type or the portal-tract type. In addition, there was no correlation between the bile duct structures and phylogenic advancement. This suggested that fish livers have developed the biliary system of vertebrates. In the biliary system, toxic substances are neutralized and eliminated in the liver. Elimination occurs in the bile, an exocrine secretion of the liver that is important for lipid digestion (Rappaport. 1963). We suggested that biliary tract structures were concerned with dietary habits, and adapted the hepatic function, including lipid metabolism.

Pleuronectiformes and Tetraodontiformes have the highest phylogenic status in Euteleostei, but their hepatocyte-sinusoidal structures have solid and tubular forms. In addition, Tetraodontiformes hepatocytes are rounded and

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store abundant neutral lipids. According to Welsch and Storch (1973), teleost livers contains two categories of hepatocytes, lipid-rich and glycogen-rich. In some species, lipid-rich cells predominate while in others glycogen-rich cells are more common (Akiyoshi *et al*., 2001). The livers of the globefish and goby have lipid-rich hepatocytes, and a well-developed biliary pathway. These findings may be characteristics relevant to special functions such as the accumulation of tetrodoxin in globefish (Narahashi, 2001). We suggested that the globefish and goby have unique livers, and show three characteristic components: (a) lipid-rich hepatocytes, (b) solid formal hepatocyte-sinusoidal structures, and (c) well-developed biliary system. In addition, the flatfish also have unique livers, and show the tubular formal hepatocyte-sinusoidal structures and a well-developed biliary pathway.

Fish are widely distributed both geographically and ecologically. Their habitats range from deep sea to small mountain streams, and from mud surfaces on land to inside holes under the seabed. Their immense diversity has created various dietary habits. The structural characteristics of their digestive organs (e.g. esophagus, stomach, intestines, livers, and pancreas) develop in order to capture, digest and absorb these requirements from their food.

This study showed that the architecture of the parenchymal arrangement was related to the phylogenic advancement, but the biliary tract structures were not involved. We suggested that biliary tract structures were concerned with dietary habits, and adapted the hepatic function, including lipid metabolism. In hepatic ontogenesis, we demonstrated that the parenchymal arrangement is formed phylogenically, but the biliary pathway may be adapted according to ecological and behavioral patterns.

ACKNOWLEDGEMENTS

We thank Mr. Hiromi Kohno and Mr. Ken Sakihara, Okinawa Regional Research Center, Tokai University, for their help in this study. We also thank Ms. Masami Matsuo and Mr. Kozo Sunada, Department of Biological Science, Shimane University, for technical assistance.

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(Received April 17, 2004 / Accepted June 3, 2004)

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