

Histology of the digestive system of the black scorpionfish *Scorpaena porcus* L.

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The present study is the first record of black scorpionfish digestive tract histology, suggesting that its histological features are mostly similar to those of other carnivorous fish and congruent to its feeding habits.

*The digestive systems of five specimens of the black scorpionfish, *Scorpaena porcus* L., from the eastern Adriatic Sea, were analysed histologically. The paraffin sections of oesophagus, stomach, intestine, liver, gallbladder and pancreas have been stained by hematoxylin – eosin technique in order to distinguish the main histological features of the digestive system in black scorpionfish. The wall of oesophagus and stomach is formed by four distinctive layers: the mucosa, the submucosa, the muscular and the outer layer. The wall of intestine and gallbladder consisted of three distinctive layers: the mucosa, the muscular and the outer layer. The mucosa is the innermost layer, which consists of two different layers: the epithelium and lamina propria. The submucosa is a layer of connective tissue with blood vessels and nerve fibres, and it has not been seen in the intestine and gallbladder. In the oesophagus, stomach and intestine, the muscular layer consists of two layers of muscle fibres: circular and longitudinal. In the oesophagus the inner muscular layer consisted of longitudinally arranged striated, while the outer layer consisted of circular muscle fibres. The arrangement of muscular layers in the stomach and intestine is reverse. The muscular layer in the gallbladder is made of a single layer of smooth muscular tissue. The outermost layer of the upper parts of visceral organs is adventitia consisting of connective tissue. In the caudal parts of digestive tract, this layer is replaced by serosa. The liver consists of hepatocytes that are surrounded by mass of fat – storing cells. The arrays of hepatocytes are separated by system of sinusoidal capillaries. The pancreas consists of serous acini.*

Key words: *Scorpaena porcus* L., digestive tract, black scorpionfish, histology

INTRODUCTION

Scorpaena porcus (Linnaeus, 1758), the black scorpionfish, is one of the most abundant and commercially important species of Scorpaenidae in the Mediterranean and the Adriatic Sea (PALLAORO & JARDAS, 1991) and the present

study provides first information on histological features of its digestive apparatus. The scorpionfish is also found along the eastern Atlantic coast, from British Isles to Senegal, around Azores and Canary Islands (HUREAU & LITVINENKO, 1986), inhabiting rocky and rocky-sandy infralittoral bottoms mostly between 5 and 15 m

depth (GRUBIŠIĆ, 1982). In the Adriatic, it attains up to 35 cm length and 0.80 kg weight (GRUBIŠIĆ, 1982).

The black scorpionfish is extensively carnivorous organisms feeding mainly on decapod crustaceans and small fish. Likewise, the decapod *Brachyura* and Pisces are its principal and preferred food, decapod *Anomura* and *Natantia* are secondary food, while the other organisms (the rest of Crustacea, Mollusca, Polychaeta) are occasional prey (PALLAORO & JARDAS, 1991).

In fish, as in other vertebrates, the alimentary tract consists of alimentary canal, a hollow tube of varying diameter which is longitudinally divided into the oesophagus, the stomach, the intestines, and the rectum (TEER *et al.*, 1995). The alimentary tract also includes some associated organs such as the tongue and the teeth in the oral cavity, and the extramural digestive organs, specifically, the liver, gallbladder, and the exocrine pancreas. The salivary glands are usually missing in the oral cavity of fish (TEER *et al.*, 1995).

Histologically, from the cranial end of the oesophagus to the caudal end of the rectum, the wall of the alimentary canal is formed by four distinctive layers. Starting at the lumen, these layers are: the mucosa, the submucosa, the muscularis externa, and the serosa or adventitia (KIERSZENBAUM, 2002).

The digestive system of fish shows remarkable diversity in its morphology and function, related to both taxonomy and different feeding habits (AL ABDULHADI, 2005).

The histological features of the digestive tract in fish in respect to differences in their feeding habits were previously studied in genera *Gobio*, *Cyprinus* and *Rutilus* (AL-HUSSAINI, 1949); in the cod *Gadus morhua* (BISHOP & ODENSE, 1966), pike *Esox lucius* (BUCKE, 1971), common eel *Anguilla anguilla* (CLARKE & WITCOMB, 1980), the seabream *Sparus aurata* (CATALDI *et al.*, 1987), amberjack *Seriola dumerili* (GRAU *et al.*, 1992), neotropical omnivorous fish (ALBRECHT *et al.*, 2001) and in damselfish *Stegastes fuscus* (CANAN *et al.*, 2012). BUDDINGTON *et al.* (1997) provided data on comparative structure and function of the intestine in carnivorous fish in relation with diet.

Although the ecological and feeding habits of the black scorpionfish have been evidenced in past (ŠOLJAN & KARLOVAC, 1932; BOUTIERE, 1958; BRADAI & BOUAIN, 1990; JARDAS & PALLAORO, 1991), as well as its biometric features (FERRI *et al.*, 2010), the aspects of its histological features of the digestive system were lacking. Therefore, the aim of this study was to describe characteristics of *Scorpaena* digestive system inferred by histology.

MATERIAL AND METHODS

A total of five black scorpionfish specimens were sampled to study histology of digestive tract. The specimens were collected in Peleš Bay near Zečevo in April 2010. Digestive tracts were immediately fixed in 12% formalin after were fish sampling. Tissues were dehydration in an ascending series of ethanol, clearing in xylene, embedded in paraffin blocks, sectioned transversally at 6 µm and mounted on glass slides. The sections were then deparaffinised in xylene, stained by hematoxylin – eosin and observed under the light microscope (Axio Imager M.1, Zeiss). Microphotographs were made by Axio Cam MRc5 Zeiss.

RESULTS

The oesophagus

Oesophagus wall contains four distinctive layers: the mucosa, the submucosa, the muscular layer and outer layer. The mucosa forms high folds protruding deeply into the oesophageal lumen. It is lined with stratified squamous epithelium. There are numerous mucous goblet cells inserted in between epithelial cells. *Lamina propria* lies just beneath the epithelium. It is composed of thick connective tissue layer containing fibrocytes and fibres (Fig. 1a). *Lamina muscularis mucosae* has not been observed. The submucosa is connective tissue layer containing blood vessels and nerve fibres. Beneath the submucosa there is *muscularis externa*, a two-layered muscular tissue. The inner layer contains longitudinally organized muscle fibres,

while the outer one consists of circular muscle fibres made of striated muscular tissue (Fig. 1b). The outermost layer is *tunica adventitia* containing loose connective tissue. No taste buds were observed in the oesophagus.

The stomach

Wall of the stomach fundus consists of four layers: *tunica mucosa*, *tunica submucosa*, *tunica muscularis* and *tunica serosa*. The mucosal surface forms shallow folds and it is lined by ciliated simple columnar epithelium (Fig. 1c). *Lamina propria* extends deeply in the mucosal folds and contains dense connective tissue and gastric glands. The gastric glands are long tubular glands containing large, polygonal cells with large nucleus. These cells could be found in the neck and base of gastric gland. Large cells, similar to mucous cells in their appearance, were observed in the base of gastric glands (Fig. 1d). The submucosa contains loose connective tissue with blood vessels and nerves. *Tunica muscularis* is a two-layered muscular tissue. The inner layer consists of the circular muscles, while the outer layer consists of longitudinal muscles. *Tunica serosa* is lined with the mesothelial layer.

Wall of the stomach pyloric region consists of four layers: *tunica mucosa*, *tunica submucosa*, *tunica muscularis* and *tunica serosa* (Fig. 1e). The mucosa is almost flat, lacking folds. It is lined by simple columnar epithelium. *Lamina propria* lies beneath epithelium and contains numerous alveolar mucous glands lined in one or two layers. Pyloric glands lie underneath mucous glands, being smaller than gastric glands in the fundic region. Pyloric glands consist of spherical cells containing small nucleus. *Tunica submucosa* is layer of loose connective tissue containing blood vessels and nerves. This layer is thinner than the counterpart layer in fundus. The main characteristic of the pyloric region is a strong circular muscle layer made of smooth muscular tissue, called pyloric sphincter (Fig. 1e). The outermost layer is *tunica serosa* made of mesothelial layer. Seven pyloric caeca are connected to the intestine after the pyloric region. Pyloric caeca consist of mucosa and two

narrow muscular layers, inner and outer. The inner layer is made of circular muscle fibres while the outer layer is made of longitudinal muscle fibres. Pyloric mucosa is a thick layer protruding deeply into the lumen. Abundance of glandular cells was seen in the mucosa, while the epithelial cells could not be properly distinguished.

The intestine

The intestine of *Scorpaena porcus* L. consists of three distinctive parts: anterior, intermediate and posterior intestine.

Anterior intestine wall is made of three layers: the mucosa, the muscle layer and the outer layer (Fig. 1f). *Tunica mucosa* forms large, prominent, protruding leafy-like folds in the lumen, lined by simple columnar epithelium covered with thick brush border and glycocalyx. The epithelial cell nucleus is basal, while few goblet cells are placed in between epithelial cells. The connective tissue of *lamina propria* enters into mucosal folds. *Lamina propria* lies directly on the muscle layer because submucosa was not observed. The muscular layer is made of two muscle fibre layers: the inner circular and the outer longitudinal layer. *Tunica serosa* is the outermost mesothelial layer.

Intermediate intestine is very thin and made of three layers: the mucosa, the muscle layer and the outer layer (Fig. 2a). The mucosa is less folded, rarely forming finger-like folds, lined by simple columnar epithelium containing microvilli. The goblet cells are placed in between epithelial cells. *Lamina propria* is a layer of loose connective tissue containing blood vessels and nerves. It lies directly on the muscle layer. The submucosa has not been observed. The muscle layer is made of smooth muscle cells arranged in two layers: the inner circular and the outer longitudinal layer. *Tunica serosa* is the outermost mesothelial layer.

The posterior part of the intestine has smaller diameter than its upper parts and is made of three layers: the mucosa, the muscle layer and the outer layer. The mucosa forms deeper folds than in the anterior and intermediate intestine.

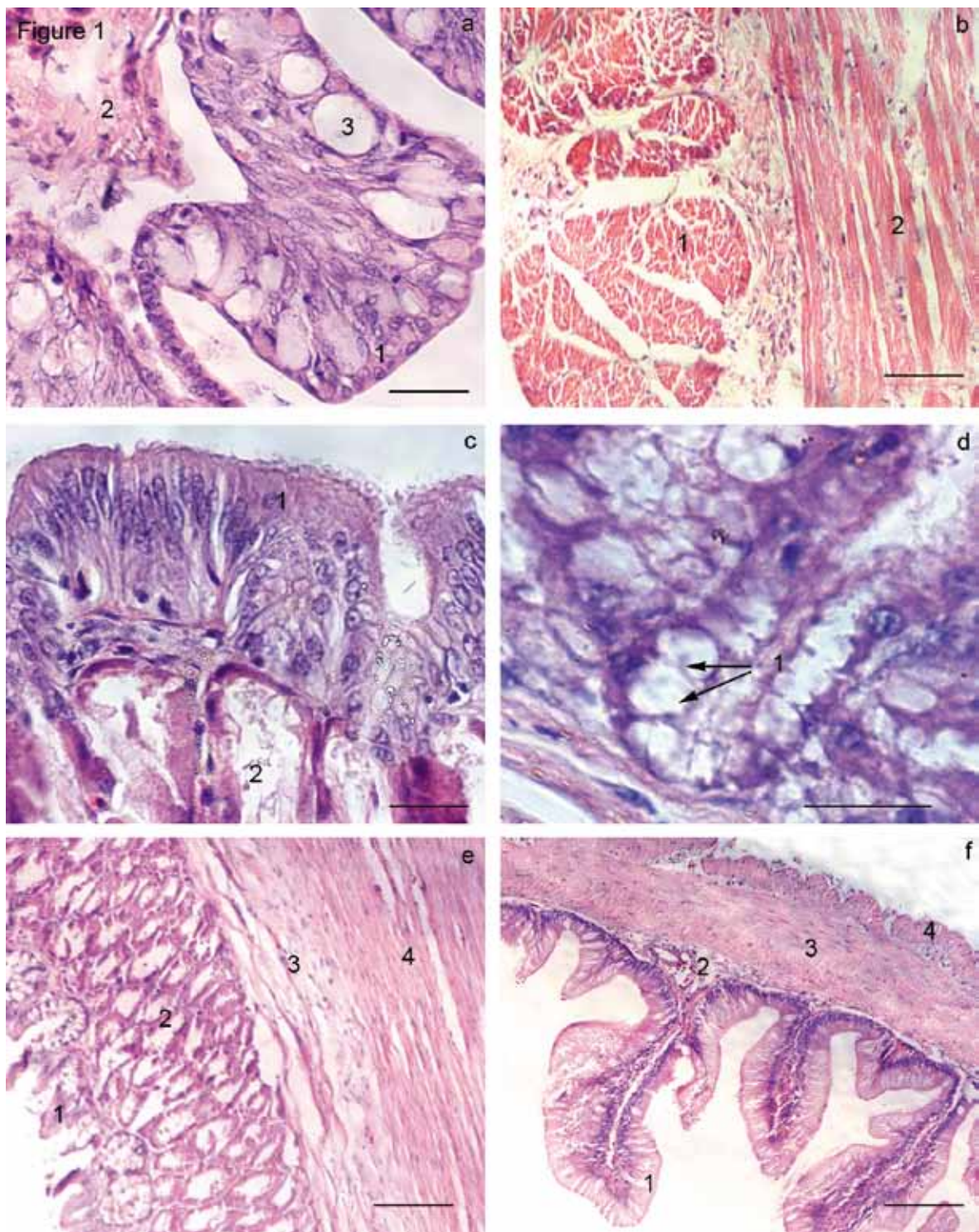


Fig. 1. **a)** Section through the *Scorpaena porcus* esophageal mucosa: 1- stratified squamous epithelium; 2 – lamina propria; 3 – goblet cell. Scale bar = 25 μm **b)** Section through the muscular layer of the oesophagus: 1- inner muscular layer; 2– outer muscular layer. Scale bar = 100 μm **c)** Section through the fundic mucosa of the stomach: 1- epithelium; 2 – apical parts of the gastric glands in lamina propria. Scale bar = 25 μm **d)** Basal part of the gastric glands: 1- numerous mucous cells. Scale bar = 25 μm **e)** Pyloric region of the stomach: 1- epithelium; 2 – lamina propria; 3 – submucosa; 4 – pyloric sphincter. Scale bar = 100 μm **f)** Section through the *Scorpaena porcus* anterior intestine: 1- epithelium; 2- lamina propria; 3 – inner muscular layer; 4 – outer muscular layer. Scale bar = 100 μm .

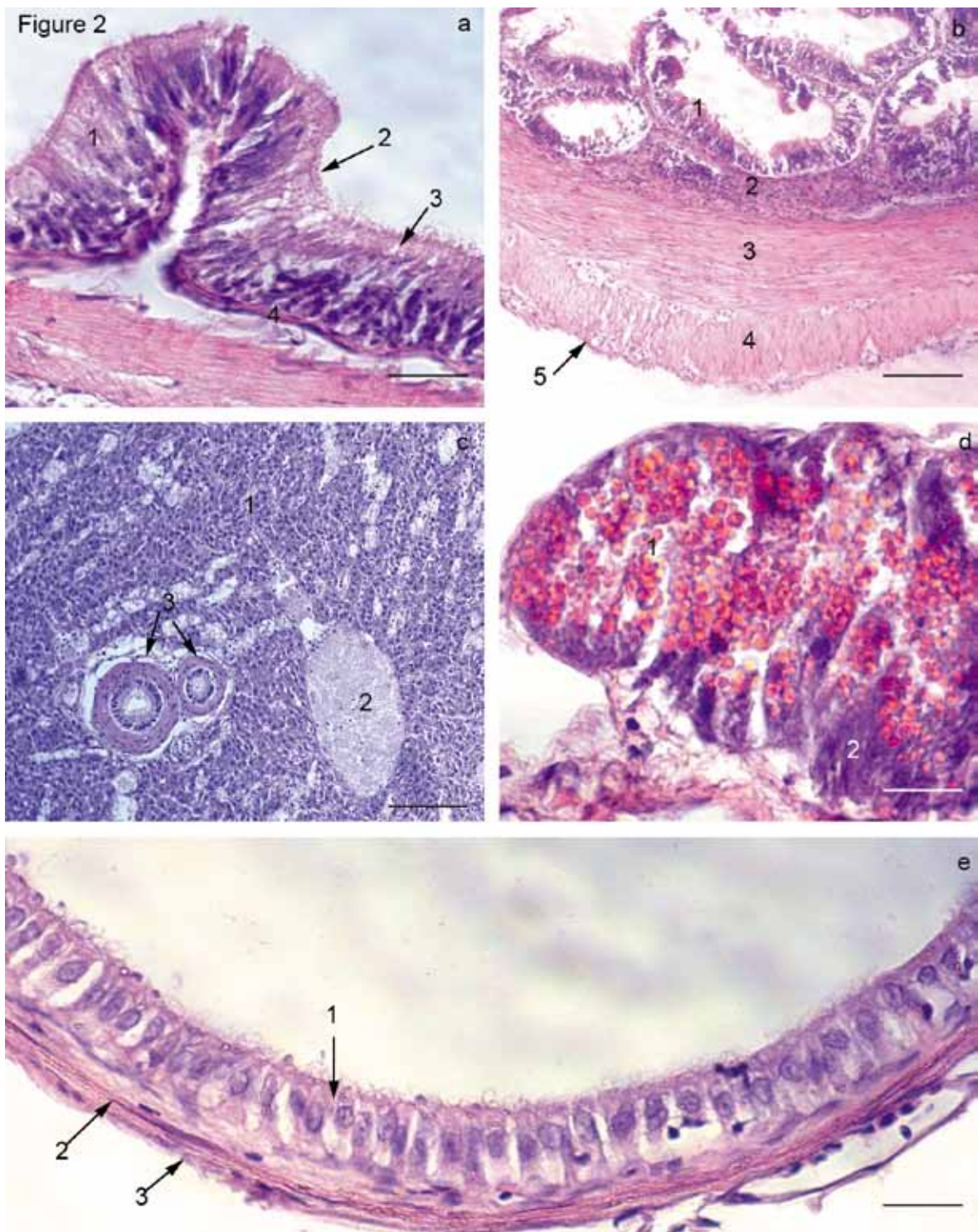


Fig. 2. **a)** Mucosa of the *Scorpaena porcus* intermediate intestine: 1- epithelium; 2 – microvilli; 3 – goblet cells; 4 – lamina propria. Scale bar = 25 μm . **b)** Section through the *Scorpaena porcus* posterior intestine: 1- epithelium; 2 - lamina propria; 3 – inner muscular layer; 4 – outer muscular layer; 5 – tunica serosa. Scale bar = 100 μm . **c)** Section through the *Scorpaena porcus* liver parenchyma: 1 – hepatocytes; 2 – middle vein; 3 – bile ducts. Scale bar = 100 μm . **d)** Section through the *Scorpaena porcus* pancreas: 1- granules in the apical parts of the serous cells; 2 - cytoplasm of the serous cells. Scale bar = 25 μm . **e)** Section through the *Scorpaena porcus* gallbladder: 1- epithelium; 2 – smooth muscle layer; 3 – tunica serosa. Scale bar = 50 μm .

Some folds are branched and mucosa seems to be reticular in structure because of oblique sectioning through very high folds (Fig. 2b). The mucosa is lined by ciliated simple columnar epithelium and goblet cells are placed in between epithelial cells. Connective tissue of *lamina propria* enters into mucosal folds. *Lamina propria* lies directly on the muscle layer. It is thicker than in the upper parts of the intestine. Some blood vessels with erythrocytes could be observed. The muscle layer is made of two layers: the inner circular and the outer longitudinal layer. The outmost mesothelial layer is *tunica serosa*.

The liver

The liver in black scorpionfish consists of two lobules. Liver cells, hepatocytes, surrounded by adipocytes were observed in hematoxylin-eosin stained sections. Hepatocytes contain large, spherical nucleus with multiple nucleoli. The nucleus is usually centrally positioned and surrounded by a narrow layer of cytoplasm. The sections through the middle veins and bile ducts could be seen in the liver parenchyma. The arrays of hepatocytes were separated by system of sinusoidal capillaries (Fig. 2c).

The pancreas

The pancreas was sectioned from the mesenteric fat between pyloric caeca and it consists of exocrine secretory units composed of serous acini. The endocrine parts were not seen. The serous acini are round or oval. The basal parts of serous cells stained basophilic and the apical part containing numerous zymogene granules stained eosinophilic. Acini opened in the blood (Fig. 2d).

The gallbladder

Gallbladder wall in the black scorpionfish is made of three layers: the mucosa, the muscle layer and the outer layer. The mucosa is lined by simple columnar epithelium. The cells have large spherical nucleus, centrally placed. Epithelial cells lie on the thin layer of *lamina propria* consisting of loose connective tissue. The mus-

le layer is made of one circular layer of smooth muscle cells. A thin *tunica serosa* is made of mesothelial layer (Fig. 2e).

DISCUSSION

The digestive system of fish is similar to the digestive tract of other animals. Carnivorous fish have short digestive tracts when compared to herbivorous fish. The alimentary canal is usually a hollow tube of varying diameter which is longitudinally divided into the oesophagus, the stomach, the intestines, and the rectum (TEER *et al.*, 1995). From the cranial end of the oesophagus to the caudal end of the rectum, the wall of the alimentary canal is usually formed by four distinctive layers. Starting at the lumen, these layers are the mucosa, the submucosa, the muscularis externa, and the serosa or adventitia (KIERSZENBAUM, 2002). In black scorpionfish, the alimentary tube opens with a big mouth containing thin and sharp teeth. Since the black scorpionfish is a carnivorous fish, its oesophagus is short, muscular tube conducting the food to the stomach. The oesophagus of different teleost fish like those of most vertebrates, function in transporting food bolus, so it is lined by stratified squamous epithelium and cells that secrete mucous that enables lubrication and extension of the organ when large prey is passed through (AL ABDULHADI, 2005). In some fish, such of genus *Gobio* and *Cyprinus*, the selection of food is based largely on taste; hence they are supplied with taste buds from lips to oesophagus (AL-HUSSAINI, 1949). On the other hand, some of them augment taste by sight and taste buds are accordingly restricted in distribution and less abundant (AL-HUSSAINI, 1949). The taste buds were not noticed in the oesophagus of the black scorpionfish. The oesophagus of the black scorpionfish is also layered by stratified squamous epithelium, abundant in goblet cells. The mucous content of these cells probably facilitates the transport of the food particles through the oesophagus.

The mucosa of the oesophagus also contains *lamina propria* and *lamina muscularis mucosae*, although the last one is usually missing in fish

(KOZARIĆ, 2001). The present study revealed that the *lamina muscularis mucosae* is also missing in the black scorpionfish oesophagus. The oesophagus of the sea bream *Sparus aurata* shows a multilayered mucosa in the upper part and single-layered regions in the lower part. The multilayered regions are formed by epithelial cells, mucus-secreting cells and by cells rich with eosinophilic granules (CATALDI *et al.*, 1987). In the common eel *Anguilla anguilla* the oesophagus mucosa consisted of stratified epithelium, columnar epithelium and goblet cells, while striated muscle fibres formed the thick muscular coat (CLARKE & WITCOMB, 1980). Two-layered *muscularis externa* of the black scorpionfish oesophagus is composed of striated muscular tissue. In fresh water stingray *Himantura signifer*, the oesophagus is covered by stratified columnar epithelium (CHATCHAVALVANICH *et al.*, 2006).

The complex morphology and histochemistry of oesophagus may indicate additional functions as it was shown in bream *Mylio cuvieri* whose oesophagus exhibits a diversity of morphologically and histochemically recognizable type of mucous cells (AL ABDULHADI, 2005).

Some fish from genus *Gobio*, *Cyprinus* and *Rutilus* are stomachless (AL-HUSSAINI, 1949). In the black scorpionfish *Scorpaena porcus* L. the stomach is layered by mucosa, submucosa, *muscularis externa* and serosa. The present study revealed that the stomach mucosa is covered by ciliated simple columnar epithelium. *Lamina propria* of the stomach contains tubular gastric glands and also some mucous cells situated on the basal surface of the gastric glands. The gastric glands are the main part of the stomach mucosa in vertebrates (KIERSZENBAUM, 2002), both in carnivorous and herbivorous fish (AL ABDULHADI, 2005). The stomach of the seabream *S. aurata* has a single-layered columnar epithelium under which, in the cardiac and fundic portion, gastric glands, comprised of similar cells, are present (CATALDI *et al.*, 1987). The pyloric region of the black scorpionfish stomach is also four-layered. The specific feature of the pyloric region is the presence of the pyloric sphincter, a strong circular muscle that enables food particles passing towards the intestine. These results

are in accordance with ALBRECHT *et al.* (2001) and CHATCHAVALVANICH *et al.* (2006).

The intestine wall of black scorpionfish is a three-layered structure containing mucosa, *muscularis externa* and serosa. Although these layers are permanently present in total length of the intestine, they differ in their morphology, so the intestine could be divided into three distinguishable parts: anterior, intermediate and posterior intestine. The main difference is based on the wall thickness and the morphology of the mucosal folds. The similar results were confirmed in some omnivorous fish (ALBRECHT *et al.*, 2001). Intestinal structure is highly variable among carnivorous fish (BUDDINGTON *et al.*, 1997). According to the published data, intestine usually consists of proximal and distal portion (AL ABDULHADI, 2005; CHATCHAVALVANICH *et al.*, 2006; DAI *et al.*, 2007). The intestines of carnivorous fish have evolved for processing nutrient dense diet that is high in protein and low in carbohydrate (BUDDINGTON *et al.*, 1997). The intestinal parts containing more complex mucosa are generally involved in absorptive processes, and the intestinal caeca located in the pyloric region increase the absorption area of the intestine (CANAN *et al.*, 2012). The posterior intestine in black scorpionfish had much narrow lumen but more complex mucosa than the two upper parts of the intestine. Accordingly, the absorption in black scorpionfish is carried out also in the lower parts of the digestive tube. The liver in fish is usually described as unilobular organ having fat storage as one of many functions (BUCKE, 1971; CLARKE & WITCOMB, 1980). The pancreas in fish could be disseminated along the upper intestine or infiltrated in liver parenchyma (CATALDI *et al.*, 1987); or unusually compact (BUCKE, 1971; CLARKE & WITCOMB, 1980). In some fish, few islets of Langerhans could be seen in pancreas (BISHOP & ODENSE, 1966; CLARKE & WITCOMB, 1980). In the black scorpionfish the pancreas was spread between pyloric caeca containing no islets of Langerhans.

The present study is the first record on black scorpionfish digestive tract histology, suggesting that its histological features are mostly similar to those of other carnivorous fish and congruent to its feeding habits.

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Histologija probavnog sustava škrpuna *Scorpaena porcus* L.

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SAŽETAK

Ovaj rad predstavlja prvi zapis o histološkoj građi probavnog sustava škrpuna i ukazuje na sličnost građe probavnog sustava s probavnim sustavom većine mesojednih riba, a u skladu s njegovim prehrambenim navikama.

Probavni sustav pet primjeraka škrpuna *Scorpaena porcus* L., iz istočnog Jadranskog mora je histološki istražen. Parafinski rezovi jednjaka, želuca, crijeva, jetre, gušterače i žučnog mjehura bojani su hemalaun – eozin tehnikom, kako bi se utvrdila osnovna histološka građa pojedinih dijelova probavnog sustava. U stijenci jednjaka i želuca škrpuna razlikuju se četiri sloja: sluznica, podsluznica, mišićni i vanjski sloj. U stijenci crijeva i žučnog mjehura razlikuju se tri sloja: sluznica, mišićni i vanjski sloj. Sluznica (*tunica mucosa*) oblaže lumen probavne cijevi i sastoji se od dva različito građena sloja: epitel (*lamina epithelialis*) i proprija (*lamina propria*). *Lamina muscularis mucosae* se ne uočava. Podsluznica (*tunica submucosa*) je sloj vezivnog tkiva u kojem su uložene veće krvne žile i živčana vlakna, a ne uočava se u stijenci crijeva i žučnog mjehura. Mišićni sloj jednjaka, želuca i crijeva sastoji se od dva sloja: kružnog i uzdužnog. U mišićnom sloju jednjaka unutrašnji sloj izgrađuju uzdužno, a vanjski kružno raspoređena mišićna vlakna, dok je u mišićnom sloju želuca i crijeva raspored mišićnih slojeva obrnut. Vanjski kružni mišićni sloj jednjaka građen je od poprečno – od prugastih mišića, dok je u ostalim dijelovima probavne cijevi mišićni sloj građen od glatkih mišićnih stanica. Mišićni sloj žučnog mjehura građen je od jednog sloja glatkih mišićnih stanica. Vanjski sloj prednjeg dijela probavne cijevi je *tunica adventitia* građena od vezivnog tkiva. U stražnjem dijelu probavnog sustava vanjski sloj čini *tunica serosa* ili ovoj peritonealnog mezotela. Jetra se sastoji od hepatocita okruženih mnoštvom masnih stanica. Između hepatocita su smještene sinusoidne kapilare. Gušterača se sastoji od seroznih acinusa.

Ključne riječi: *Scorpaena porcus* L., probavni sustav, škrpun, histologija

